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Many ways, one microorganism: Several approaches to study *Malassezia* in interactions with model hosts

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Abstract

Malassezia, a lipophilic and lipid-dependent yeast, is a microorganism of current interest to mycobiologists because of its role as a commensal or pathogen in health conditions such as dermatological diseases, fungemia, and, as discovered recently, cancer and certain neurological disorders. Various novel approaches in the study of *Malassezia* have led to increased knowledge of the cellular and molecular mechanisms of this yeast. However, additional efforts are needed for more comprehensive understanding of the behavior of *Malassezia* in interactions with the host. This article reviews advances useful in the experimental field for *Malassezia*.

Introduction

The *Malassezia* genus was discovered in the early 19th century. Currently, 18 species belonging to this genus have been identified, all of which are considered to be important contributors to the human and animal mycobiota [1,2]. In addition to the 18 current species, the incorporation of *M. auris*, *M. palmae* and *M. rara* has been recently proposed [1]. *Malassezia* yeast can cause some dermatological conditions, such as pityriasis versicolor, atopic dermatitis, dandruff, seborrheic dermatitis, and folliculitis but also plays a role in systemic infections. Recently, *Malassezia* has been associated with chronic diseases such as inflammatory bowel disease (IBD), cancer, and neurological disorders such as Alzheimer's disease [3]. These findings continue to attract attention and emphasize the importance of understanding the role of this microorganism in human health.

Experimental approaches allow the study of this yeast at the cellular and molecular level. In addition, genomic data from multiple species are now available, allowing the identification of *Malassezia* genes that are involved in environmental adaptation, metabolism, pathogenicity, and antifungal resistance [4]. The use of a convenient system of genetic manipulation through *Agrobacterium tumefaciens*–mediated transformation (AMT) and a CRISPR/Cas9 system could increase the ability to study gene function and provide information about pathogenicity [4].

Given its role in the human mycobiota, it is important to understand how *Malassezia* interacts with the host. Both in vitro (ex vivo) and in vivo models have been studied, as summarized in Table 1. An in vivo mammalian skin model using C57BL/6 mice, which exhibits similarities to the human immune system, facilitated the elucidation of a key role of *Malassezia* in inducing a Th17 response associated with exacerbation of skin inflammation [5]. In addition, a murine model has been used to study the role of *Malassezia* in IBD and the pathogenesis of pancreatic ductal adenocarcinoma (PDA). Interestingly, a mouse model led to the finding that *M. restricta* has a negative association with Huntington's disease [5–8]. Unfortunately, a murine model also has disadvantages, including ethical concerns and high cost. However, invertebrate models, such as *Galleria mellonella*, may be alternatives, offering advantages such as easy management, low cost, and similar immune response to humans [9]. In addition, ex vivo models (for instance, skin explants) and in vitro models (for instance, keratinocyte cell lines and reconstructed human epidermis) provide conditions similar to the human skin to study the human immune response [10–12].

The combination of various strategies and tools is necessary to further deduce *Malassezia*–host interactions (Fig 1). Omics approaches, such as lipidomics and volatilomics [28,29], may potentially lead to a deeper understanding of the genus by identifying factors associated with its commensal and pathogenic status. Also, considering the emergence of antifungal resistance in this genus, this could facilitate the development of therapeutic strategies that modulate the homeostasis of the yeast without disruption to the host.

Undoubtedly, research on *Malassezia* is still young, underscoring the importance of understanding the lipid requirements of this genus to determine the ideal conditions that allow for the survival of *Malassezia* in the niches that they occupy. In this review, we highlight advances in the field and different strategies that can be used to continue to learn about this genus and its interactions with hosts.

Beyond what you can see: Is it possible to study *Malassezia* interactions with a model host?

The implementation of microscopy-based techniques is a new field in *Malassezia* research, especially when the goal is to evaluate the interaction between this yeast and its host. Various microscopy-based techniques [30] in combination with other approaches can be implemented to understand whether *Malassezia* can invade the tissue or if the disease is caused by other factors and to document the interaction between *Malassezia* and model hosts.

Microscopy-based techniques can be used to analyze the progression of infection, invasion of cells, colonization, and biofilm formation of *Malassezia* with in vivo or ex vivo models (Table 1). For example, Corzo-León and colleagues used various stains (periodic acid solution, Schiff reagent, counterstain with hematoxylin solution) and microscopic techniques (fluorescent confocal microscopy [FCM] and SEM) to reveal the host–pathogen interaction in oily and non-oily skin in an ex vivo human skin model infected with *M. sympodialis* [10]. Used together, these techniques demonstrated a direct interaction between *Malassezia* and keratinocytes and the epidermal damage caused by these fungi [10]. In addition, histological stains have been used to evaluate yeast–host interactions and the cellular response to infection, tissue invasion, and colonization in *G. mellonella* and RhE models infected with *Malassezia* [9,12]. Further, FCM has been standardized for costained *Malassezia* and lipid storage organelles (i.e., lipid droplets), which are promoters of pathogenicity and survival during stress conditions in other microorganism models [31].

Microscopy techniques are widely used in other pathogenic fungi to investigate tissue invasion, cell damage, and the host–pathogen interaction. For example, fluorescent imaging

Table 1. Different studies applying experimental models to study *Malassezia*–host interactions.

| Current models to study host–microbe interaction				Studies performed in *Malassezia* species		
Model category	Experimental model	Advantages	Disadvantages	*Malassezia* species	Methodological assessment/parameters analyzed	Findings
Invertebrates	*Galleria mellonella*	• Conserved innate immune system • Low cost • No ethical implications • Minimal infrastructure requirements • Easy management [9]	• No adaptive immune response • No fully sequenced genome [13,14]	*M. furfur* *M. pachydermatis*	• Histological stain: H&E • Confocal microscopy • Larval melanization and survival • Fungal burden • Hemocyte response	*G. mellonella* is a suitable model to evaluate the *Malassezia*–host interaction process, where the survival of larvae is dependent on inoculum concentration, species of *Malassezia*, and incubation temperature [9]
	Drosophila melanogaster	• Short life cycle • Low cost • Genome available • Available advanced technologies to apply • Conserved innate immune system [15]	• No adaptive immune response [13]	*M. pachydermatis*	• Histological stain: HE, GMS • Fly survival • Fungal burden • Systemic infection	Toll-deficient flies infected with *M. pachydermatis* are susceptible to infection, and they are inoculum dependent compared to wild type [16]
	Caenorhabditis elegans	• Short life cycle • Physiologically simple • Genome available • Transparent cuticle • Easy to obtain and manage [14]	• No adaptive immune response • Unable to be cultured at 37°C • Difficult to inoculate [14]	*M. pachydermatis*	• Worm survival	*C. elegans* showed a high mortality after 96 h of exposure to plates incubated with *M. pachydermatis* at 25°C [17]
Vertebrates	Mouse	• Conserved immune response • Various routes of fungal administration (intravenous, cutaneous, ocular, vaginal, intragastric, oropharyngeal) • Development of systemic symptoms of infection • Sequenced genome [13,18]	• High cost • Ethical implications • Special requirements (larger space, optimal asepsis) • Experience and training are needed [13]	*M. pachydermatis*	• Histological stain: HE, methenamine silver stain • Fungal burden	*M. pachydermatis* causes otitis and dermatitis in mice, with a high burden at the beginning of infection that decreases over time [19]
				M. sympodialis	• RT-qPCR • FISH	Coinfection of mice with *M. sympodialis* and *Pseudomonas aeruginosa* or *Staphylococcus aureus* influence the immune response of the host [20]
				M. pachydermatis *M. furfur* *M. sympodialis*	• RT-qPCR • Histochemistry • Immunofluorescence • Flow cytometry and cell sorting • Histological stain: HE, periodic acid-Schiff	*Malassezia*-induced IL-17 immune response in the skin results in fungal reduction and promotes inflammation [5].
				M. restricta	• Morphological evaluation • Histological stain: HE • Flow cytometry • qPCR	The presence of *M. restricta* did not affect the mouse colon but exacerbated DSS-induced colitis. *M. restricta* led to severe intestinal inflammation with higher production of IL-17A- and IFN-γ-producing CD4+ cells [6]
				M. restricta	• Shotgun metagenomic sequencing	*M. restricta* was a key species in the gut microbiome with a negative association with Huntington's disease in the R6/1 transgenic mouse model [8]
				Malassezia spp. *M. globosa*	• qPCR • Histological stain: HE, Gomori trichrome • IHC and microscopy • FISH • DNA sequencing	*Malassezia* spp. can migrate from the gut to the pancreas, and its presence there is higher in mice with PDA. This may be mediated by activation of the MBL pathway [7]

(*Continued*)

Table 1. (Continued)

	Current models to study host–microbe interaction			Studies performed in *Malassezia* species		
Model category	Experimental model	Advantages	Disadvantages	*Malassezia* species	Methodological assessment/parameters analyzed	Findings
				Malassezia furfur	• Histological stain: HE, periodic acid–Schiff staining • Primary keratinocyte cell culture • RT-qPCR • Antibody treatment (to neutralize the IL-36 receptor) • Flow cytometry • Cytotoxicity assay • Epicutaneous infection in mice • Fungal burden	Mice inoculated with *M. furfur* show hyperkeratosis and epidermal thickening. *M. furfur* triggers a IL17 immune response mediated by the IL-36 receptor through expression of IL17-associated molecules in an epicutaneous mice model, with implications for skin inflammation induced by *Malassezia* infection [21].
In vitro	In vitro cell lines (keratinocytes)	• Easy to manage • Low cost • No ethical implications • Reproducible results • Deep knowledge of cell lines [22]	• Genetically modified cell lines change phenotype and functions • Short observation time • Results cannot be interpolated with in vivo models [22].	*M. furfur*	• RT-qPCR • Western blot • ELISA • Cell viability • Negative-stain TEM • Confocal microscopy • IHC	Nanoparticles produced by *M. furfur* are internalized into the HaCaT cell line and stimulate IL-6 production [11]
				M. furfur *M. globosa* *M. obtusa* *M. restricta* *M. slooffiae* *M. sympodialis*	• ELISA • TEM	Removing the *Malassezia* capsular-like layer triggers a significant increase in the production of IL-6, IL-8, and IL-1a and a decrease in intracellular IL-10 in keratinocytes [23]
				M. pachydermatis	• Histological stain: Wright's stain • RT-qPCR • Invasion assay	*M. pachydermatis* can invade HaCat cells and triggers a strong cellular response [24]
	Ex vivo model[a]	• Isolated tissue closely mimics natural tissue conditions in the in vivo model [25]	• Technically demanding • Short observation time [25]	*M. sympodialis*	• SEM • Confocal microscopy • Histology: HE, periodic acid–Schiff staining • RT-qPCR • Immunoassays • Proteomics	Oleic acid in the skin is associated with direct contact of yeast and keratinocytes, as well as damage to the epidermis. The skin exposed to *Malassezia* in oily conditions expresses IL-18 but not antimicrobial peptide genes [10]
	RHE	• Possibility of incorporating various cell types in combination with keratinocytes • No ethical implications • Reproducible results • Higher degree of standardization [26,27]	• Impairment of barrier function • Lack of equivalent for dermal features (for instance, vasculature, glands, and lipids) • Short observation time • No adaptive immune response [27]	*M. furfur* *M. sympodialis*	• Light microscopy • Wide-field fluorescence microscopy • SEM • Cytotoxicity assay • RT-qPCR	*M. furfur* and *M.sympodialis* colonize and form biofilm at the RhE surface [12]

FISH, fluorescence in situ hybridization; GMS, Grocott Gomori methenamine-silver nitrate; HE, hematoxylin–eosin; IHC, immunohistochemistry; MBL, mannose-binding lectin; PDA, pancreatic ductal adenocarcinoma; RhE, reconstructed human epidermis; RT-qPCR, quantitative reverse transcription PCR; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

[a]Explanted organs such as skin explant.

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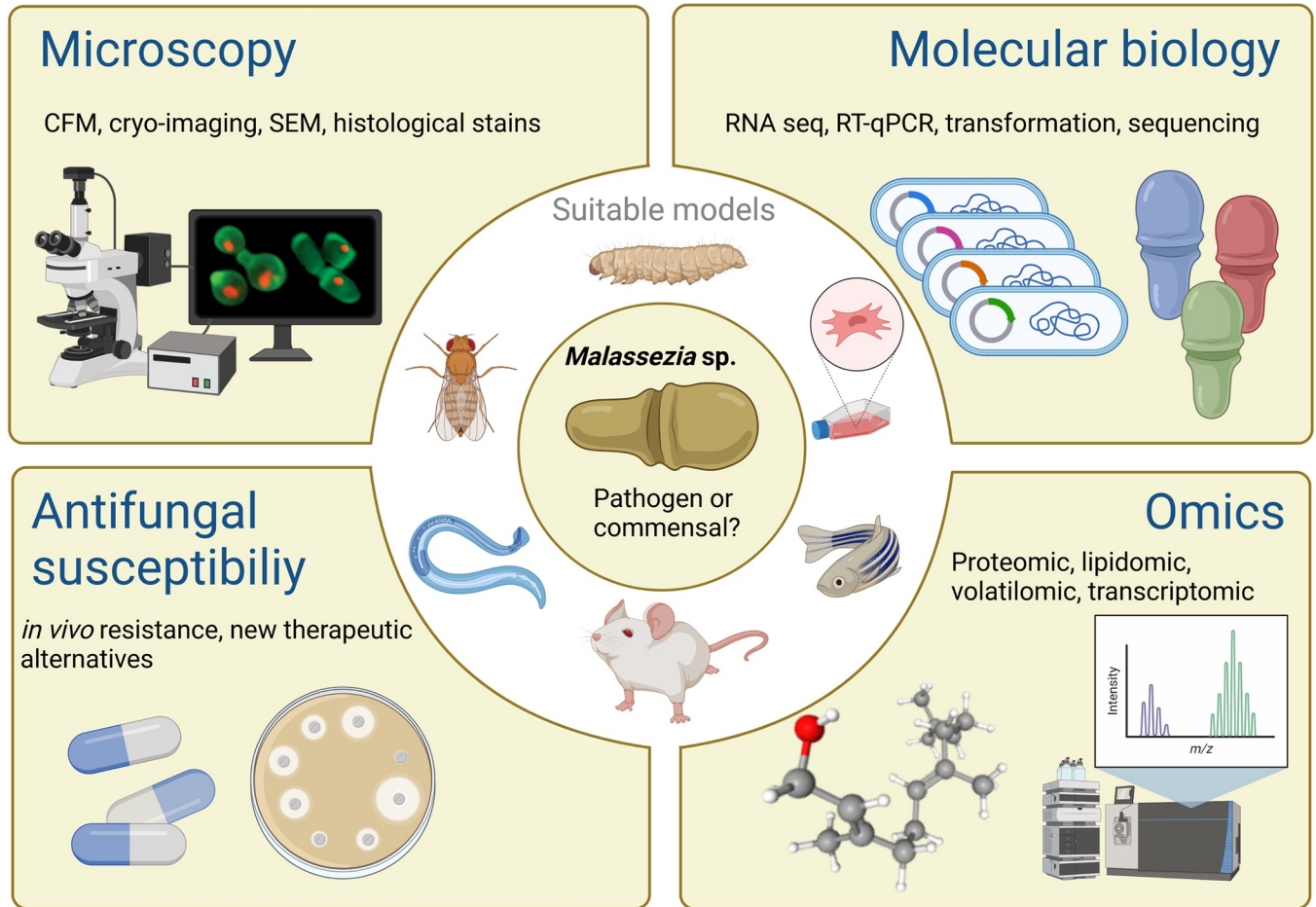
Fig 1. Approaches to study the pathogenicity of *Malassezia* spp. Various in vivo and in vitro models allow the study of infection processes and the evaluation of the host cellular and molecular responses. Multiple methodologies can be used together in experimental models to better understand *Malassezia* metabolism and implications in pathogenicity. The image was designed in Biorender.com. FCM, Fluorescence confocal microscopy; RT-qPCR, quantitative reverse transcription PCR; SEM, scanning electron microscopy.

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methodology was used to visualize the interaction of macrophage cells in zebrafish and *Cryptococcus neoformans* [32]. In this study, Bojarczuk and colleagues established that macrophages could control infection in zebrafish and observed infection progression in detail [32]. Likewise, zebrafish as a model host for fungal infections has also been used in *Candida albicans* because it allows prolonged in vivo imaging of host–pathogen interactions, especially for bloodstream infections [33]. A bronchoscopic fibered confocal fluorescence microscopy (FCFM) technique was implemented and standardized for in vivo visualization and monitoring of *Cryptococcus* and *Aspergillus* infections in murine lungs [34]. This FCFM technique enabled researchers to visualize and describe morphological features of fungal cells during in vivo infection, which provided insight into how the condition progresses [34]. Implementing these techniques to visualize *Malassezia* in the skin could help to better understand how infection develops, the possible physical changes that occur to the yeast in the evolution of disease, and the relationship between the yeast and host macrophages or other cells.

FCM and cryo-imaging techniques have been used to describe the cellular response of the infection of *Aspergillus fumigatus* in *G. mellonella* [35]. The cryo-images showed nodule development demonstrating dissemination and melanization indicating tissue invasion [35]. FCM

of the nodules confirmed the presence of germinating conidia and hyphae [35]. In combination with other techniques, these approaches could be implemented for *Malassezia* in different host models to answer questions related to physical interactions, dissemination, and invasion that can help to determine whether *Malassezia* is a commensalist or pathogen.

Focusing on the extraordinary: How could infection models help to determine the unknown function of *Malassezia* genes and the role of gene products in virulence?

In *Malassezia*, variability in virulence has been reported within species and between species [36]. However, it is unclear which genes are involved in this variability and their role in *Malassezia* pathogenesis, homeostasis, or host interactions. At least 44 *Malassezia*-specific gene clusters exist, some likely acquired through horizontal gene transfer. However, most have unknown functions [37]. The linkage between the mating loci in *Malassezia* may be involved in pathogenesis variability [37].

Assessing the function of genes can be challenging due to the biological features of the yeasts belonging to the *Malassezia* genus, including lipid requirements, feasibility of cell contact during transformation processes, growing time, and the cell wall structure [38]. Thus, development of a transformation technique that overcomes these challenging features is much needed for gene function studies.

A. tumefaciens–mediated transformation provides random insertional mutagenesis and CRISPR/Cas9-mediated targeted gene deletion in *Malassezia* yeasts [38–41]. This tool, in combination with a murine model and macrophages as an ex vivo model, has facilitated the evaluation of the role of *M. sympodialis* flavohemoglobin, a protein encoded by a horizontally transferred gene [42]. In both models, flavohemoglobin was not necessary to establish infection in the murine model or for survival inside macrophages [42]. This is the first approach to study *Malassezia* gene function in host–microbe interactions, and more studies are needed.

In addition, *A. tumefaciens*–mediated transformation and CRISPR/Cas9–mediated targeted gene deletion can be used to study the mechanism of action of therapeutic strategies, such as calcineurin inhibitors [43]. However, given the large number of genes involved in the *Malassezia* host–microbe interaction and the relative lack of data on these genes, in vivo models are needed. Larvae of *G. mellonella* and zebrafish may serve as alternative in vivo systemic infection models. In addition, superficial and systemic infection models in adult zebrafish may provide opportunities to assess virulence and gene function [13,33]. Amorim-Vaz and colleagues evaluated 22 targeted transcription factor mutants of unannotated genes of *C. albicans* in *G. mellonella*, which demonstrated the reliability of this insect as a fungal infection model with results that correlated with a murine model [44]. Similarly, García-Carnero and colleagues demonstrated the reliability of *G. mellonella* larva as a model for *Candida* spp. and mutants with reduced virulence. This study identified predictors of virulence, such as changes in hemocyte circulation, melanization, phenoloxidase, and lactate dehydrogenase activity [45]. A 2-day postfertilization zebrafish larvae systemic infection model has allowed in vivo assessment of the infection process and tissue invasion with *C. neoformans*, a species more closely related to *Malassezia* spp. than *C. albicans*, with results that correlate with previous observations of the host innate immune response in the murine model [46]. Zebrafish larvae inoculated with *C. neoformans* mCherry-expressing deficient mutants and wild-type strains were observed with fluorescence microscopy, demonstrating (i) the mechanism of immune control of the infection and (ii) its ability to survive phagocytosis and invade tissues [46]. These examples demonstrate the feasibility of this experimental design to identify the function of *Malassezia* genes and their role in virulence.

The zebrafish larval infection model is a well-known animal model that is amenable to genetic modification. In addition, this model can be implemented in combination with the *A. tumefaciens*–mediated transformation technique to observe in vivo changes in cellular interaction associated with the genes of interest. This may lead to a better understanding of the *Malassezia*–host interaction.

Comprehending the complex metabolism of *Malassezia*: Is the metabolism of *Malassezia* related to pathogenic processes?

The complex metabolism of *Malassezia* results in the production of different molecules or metabolites that could be involved in the transition from commensal to pathogenic behavior. These molecules include lipids, proteins, and volatile organic compounds (VOCs), knowledge of which could increase the understanding of the pathogenicity of *Malassezia* species, as has been described for other microorganisms [47–49].

The production and assimilation of lipids in yeasts have been studied due to their involvement in membrane composition and their role in regulating cell membrane–associated proteins [50]. The study of lipid metabolism in *Malassezia* is relevant because of the importance of lipids in energy storage, signaling processes, metabolism, and membrane composition [31,47], as well as the fact that *Malassezia* is lipid dependent. Lipid characterization has shown differences between species during the stationary phase, revealing 18 lipid classes and 428 lipidic compounds. These lipids are represented by sterols, triacylglycerols, diglycerides, and fatty acid esters of hydroxy fatty acids. Curiously, the compounds' concentrations vary between species. For example, the content of cholesteryl ester is lower than other lipid classes (for instance, cholesterol or triacylglycerols) in *M. furfur*, atypical *M. furfur*, and *M. pachydermatis* and undetectable in *M. globosa*, *M. restricta*, and *M. sympodialis* [51]. Moreover, lipidomic and proteomic analyses identified lipid metabolism proteins, most of which are enzymes involved in lipid biosynthesis. The complex lipid metabolism of *Malassezia* may contribute to the genus's pathogenic processes [51,52]. Other assessments have revealed a connection between the production of lipid mediators in human skin and *Malassezia*, raising questions about the role of these lipids in the establishment of disease [28]. The role of lipids in disease development has been described in a *Saccharomyces cerevisiae* model, in which disturbances in cellular lipid homeostasis resulted in cell death induced by free fatty acid toxicity and lipid peroxidation in the mitochondrial pathways of apoptosis [47]. In *C. albicans*, phospholipid pathways enhance virulence; for example, the phosphatidylserine synthase mutant is avirulent in mice and has reduced production of phosphatidylethanolamine, which is thought to be involved in cell wall integrity, mitochondrial function, and filamentous growth [53]. In addition, lipidomic analysis enables the study of lipid composition under specific conditions. For example, lipidomic characterization of *Fusarium oxysporum* isolates infecting *G. mellonella* revealed a higher number of phospholipid species with higher unsaturation in clinical versus environmental isolates [54].

Proteins are important in metabolism because they are functional molecules that perform biochemical reactions due to transcriptomic processes [55]. Proteomic analyses can provide information about cell biology, host–pathogen interactions, antimicrobial resistance, biomarker discovery, and identification of anti- and propathogenic cellular responses [55,56]. Few studies have been performed to unravel the role of *Malassezia* proteins in host interactions. One of these involved protein characterization using liquid chromatography with tandem mass spectrometry and found that human skin exposed to *M. sympodialis* had increased protein expression by 18%. The proteins reported in that study were mainly related to cornification, antimicrobial immune response, and defense response to fungus [10]. An in vitro

study assessed the effect of *M. globosa* aspartyl protease 1 (MgSAP1) on *S. aureus* biofilm production and found that MgSAP1 could cleave the *S. aureus* protein A, which is involved in biofilm production [57], demonstrating a possible protective role of *M. globosa* on the skin. Still, it is necessary to evaluate this activity in ex vivo or in vivo models to better understand the effect of this aspartyl protease on a potential pathogen.

Host–pathogen interactions have been assessed via proteomic approaches in other microorganisms. For example, protein expression during *A. fumigatus* infection of *G. mellonella* revealed increased levels of antimicrobial peptides and proteins that contribute to the innate immune response to fungus in mammals [35]. Similarly, in a *C. albicans*–infected G. *mellonella* model, the yeast secreted several proteins related to pathogenesis, oxidative stress, hyphal cell wall formation, and heat shock into the larval hemolymph, which enhances the *C. albicans* virulence process [48]. These methodological approaches could be applied to the *Malassezia*–host interaction to identify proteins involved in host interactions, those related to virulence, and those that interact with the host immune response.

Fungal volatiles are gaining relevance because of their involvement in host–pathogen interactions and use as biocontrol alternatives [58]. A recent study detected 61 VOCs in different growth media supplemented with lipids in the *M. furfur* exponential and stationary growth phases [49]. This study confirmed chemical differentiation of the VOCs under other conditions. For instance, γ-dodecalactone was identified in the modified Dixon and oleic acid media in both growth phases, but not in palmitic acid or the combination oleic–palmitic acid media. In addition, differences were observed in the production of VOCs according to the growth phase. For instance, dimethyl sulfide increased during the stationary phase, while others, including octane, decreased, suggesting that *Malassezia* VOC production is stimulated by the compounds in the growth media and demonstrating the dynamic metabolism of this yeast [49]. However, no information exists for other species regarding VOC production, function in metabolism, or role in biological interactions. In contrast, the role of VOCs produced by several microorganisms has been described. Gas chromatography with mass spectrometry has demonstrated that VOCs produced by the pathogen *A. fumigatus* are toxic to *D. melanogaster* on the basis of fungal VOCs interrupting the metamorphic development of the insect. Further, compounds such as 1-octen-3-ol and isopentyl alcohol may increase the pathogenicity of the fungus [58]. In *G. mellonella*, a coinfection model demonstrated that sulfur compounds produced by *P. aeruginosa* promoted the growth of *A. fumigatus* [59]. These findings may help to understand the role of metabolism in virulence during host–pathogen interactions.

Understanding resistance in *Malassezia*

The study of antifungal resistance in *Malassezia* is of clinical interest because *Malassezia* spp. have been identified as the etiologic agents of bloodstream infections and are associated with severe systemic disease [60–62]. Considering the recurrence of *Malassezia* skin disease and the need for long-term antifungal treatment, precautions are needed to prevent the emergence of resistance to various classes of antifungal agents [62,63]. However, due to the nutritional requirements of this genus, it has been difficult to standardize interlaboratory methodologies for in vivo and in vitro analyses [64]. As such, several groups have modified the in vitro Clinical and Laboratory Standards Institute M27-A3 and European Committee on Antimicrobial Susceptibility Testing protocols by adjusting temperature and supplementing culture medium with different lipids [65]. Nonetheless, it is important to note that there is no common agreement in a standard methodology as there is for pathogenic yeasts such as *Candida* and *Cryptococcus*, and the lack of clinical cutoff values hinders the determination of resistance [65].

Recent studies have reported isolates from different *Malassezia* spp. with high minimum inhibitory concentrations (MICs) against some azoles, such as fluconazole, voriconazole, and ketoconazole [62,66,67]. Given the growing evidence of the emergence of azole resistance in *Malassezia*, there is increasing interest in understanding the mechanisms behind this phenomenon. Hence, various approaches have been used to understand resistance in *Malassezia*, including (i) synergism studies in which combinations of azoles and efflux pump inhibitors like haloperidol, pro-methazine, and cyclosporine A reduced azole MICs compared to treatment without inhibitors in *M. furfur* and *M. pachydermatis* [68]; (ii) genomic studies in which ketoconazole resistance in a *M. pachydermatis* isolate was attributed to duplication of genes encoding ERG4 and ERG11 [69] and findings that duplication of ATM1 and ERG11 could explain resistance to this azole in *M. restricta* [67]; and (iii) gene expression and RNA-seq analyses that demonstrated that efflux pumps such as PDR5 [67] and PDR10 [61] are up-regulated in resistant isolates of *M. restricta* and *M. furfur*, respectively, exposed to azoles (ketoconazole and clotrimazole). The latter was also confirmed via CRISPR-Cas9 deletion of PDR10. These results demonstrated this efflux pump provides azole resistance in *M. furfur* and is not only attributed to CYP51 mutations as reported previously [61].

Although the use of antifungal agent susceptibility profiling is increasing *Malassezia*, much remains unknown about the in vivo course of antifungal treatment. Mammalian models are the standard to evaluate the efficacy and pharmacokinetics of novel and traditional antimicrobial agents [70]. Regardless, there is a growing interest in using other models of infection, such as *G. mellonella*, considering ethical issues with mammalian models [71,72]. The *G. mellonella* invertebrate model has been used in different infection models to study pharmacokinetics due to similar responses found in humans [70,72]. Although *Malassezia* infections have been successfully established in murine models and *G. mellonella*, no antifungal activity assays have been performed [9,73]. Thus, in vivo models and methodologies that allow the evaluation of novel antifungal or alternative therapeutic treatments are needed [64].

Considering that antifungal susceptibility tests have been performed on other yeasts and filamentous fungi in a wide variety of experimental models, background studies support standardization of a model for *Malassezia*. For instance, 2 antifungal compounds have been evaluated in a coculture of *Trichophyton rubrum* and keratinocytes in which the expression of genes related to therapeutic targets and resistance mechanisms was determined by quantitative RT-PCR [74]. In addition, *G. mellonella* has been used to verify an in vitro–in vivo correlation of the combined effect of antifungal treatments on Mucorales growth [75]. As another example, a zebrafish model was proposed for antifungal compound screening in *C. albicans* infection [76]. Likewise, murine models have been used to determine the activity of new molecules, such as occidiofungin and T-2307, against *C. albicans* and *C. neoformans*, respectively, with a correlation between in vitro and in vivo results [77,78].

New therapeutic candidates and alternatives have been proposed for *Malassezia*-associated infections. For instance, L-lysine [79], AMP, essential oils, and plant extracts [80] have been proposed as alternatives to traditional antifungals therapies, but further research is needed to study their effectiveness in vivo [79]. It is crucial to standardize models to evaluate the efficacy, safety, and pharmacokinetics of traditional and alternative therapies and to corroborate whether there is a correlation between in vivo and in vitro antifungal efficacy.

Conclusions

Considering the efficacy of current techniques applied to in vivo and in vitro models to understand host–pathogen relationships and aspects related to metabolism in other fungal species, it would be worthwhile to introduce these tools to the study of *Malassezia*. These models can be

used to inform the application of strategies to identify the role of different molecules in host interactions. Even identification of the pathways by which these metabolites are produced could clarify the behavior of *Malassezia*. Such studies will make it possible to propose alternative therapeutic targets to control infections caused by these yeasts or improve diagnostic techniques.

Acknowledgments

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