

REVIEW

The human mycobiome in health and disease

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Abstract

The mycobiome, referring primarily to the fungal biota in an environment, is an important component of the human microbiome. Despite its importance, it has remained understudied. New culture-independent approaches to determine microbial diversity, such as next-generation sequencing methods, are greatly broadening our view of fungal importance. An integrative analysis of current studies shows that different body sites harbor specific fungal populations, and that diverse mycobiome patterns are associated with various diseases. By interfacing with other biomes, as well as with the host, the mycobiome probably contributes to the progression of fungus-associated diseases and plays an important role in health and disease.

Introduction

Studies of fungi have been ongoing for more than 150 years, beginning with the first mycological paper published in 1852 [1,2]. It is only in the past 10 years, however, that the microbiome as a novel microbiological concept, in which all microbes (bacteria, archaea, viruses and fungi) in an environment are taken into account, has drawn closer attention [3]. In particular, two large government-backed projects launched in the past five years - the Human Microbiome Project (HMP) in the United States and Metagenomics of the Human Intestinal Tract (MetaHit) in Europe - have provided progress in this new field of research [4-6]. These projects have defined what constitutes the normal bacterial microbiome of various human body sites, such as the oral cavity, skin, vagina, and gut. Exciting as the results have been, the characterization of fungal diversity in these body sites is still lacking. In fact, the word 'microbiome' has implied reference to only commensal and pathogenic

bacteria [7]. This connotation changed in 2010, when the term 'mycobiome' (a combination of the words 'mycology' and 'microbiome') was first used to refer to the fungal microbiome [8]. Still, in a recent search of PubMed (6 July 2013), the term 'mycobiome' appeared in only 10 publications, and relevant studies - with or without using this specific word - numbered fewer than 40. Clearly, this field is still in its infancy.

There are several reasons to include the mycobiome in assessments of the biota of specific environments. First, the incidence of fungal infection has increased greatly in the past two decades, primarily in opportunistic infections of immunosuppressed populations, such as in those who have AIDS or have undergone organ transplantation or cancer chemotherapy [9]. Second, several diseases that were formerly considered to have no association with fungi, such as hepatitis B [10], cystic fibrosis [11,12] and inflammatory bowel disease (IBD) [13,14], are now found to be associated with particular mycobiomes. Finally, the interaction between different biomes [15], and between the host and the mycobiome [16], are critical in disease progression. In this review, we summarize the methodology used to study the mycobiome, its composition and role in health and disease, and the outlook for this field.

Tools used to explore the fungal biome

The most basic fungal culture technique, dating back to the 1920s, involved growing fungal mats in flasks of sterile liquid media. The experimental results were evaluated by looking at the growth structures and assessing their composition within the media [17]. Fungal culture methodology has improved over the decades, but even now, organisms that occur in low abundance and those that require microbe-microbe interactions to grow cannot be cultivated optimally [18].

Like unculturable bacteria in the microbiome, unculturable fungi comprise the largest part of the human mycobiome. In a study of the bacterial and fungal microbiome of patients with cystic fibrosis, more than 60% of the species or genera were not detected by culture [19]. A study focusing on the mycobiome of the oral cavity reported that 11 of the 85 fungal genera identified could not be cultured [6]. In the gastrointestinal mycobiome, culture-independent methods identified 37

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different fungal groups compared to only 5 species found by culture-dependent analyses [10].

The limitations of culture-dependent methods for mycobiome studies have led to the introduction, over the past 20 years, of culture-independent approaches. Methods for classifying fungi that do not rely on microbial culture include restriction fragment length polymorphism (RFLP) analysis, oligonucleotide fingerprinting of rRNA genes (OFRG), denaturing gradient gel electrophoresis (DGGE), and *in-situ* hybridization (Table 1). These techniques are useful for comparing fungal diversities between different groups, but they lack the specificity necessary to identify the different fungal species in a large-scale study.

Direct sequencing of fungal genes has proven to be the most efficient method for classifying the mycobiome. Furthermore, with the development of next-generation sequencing (NGS) platforms, direct sequencing has become more cost-effective than was the case when only classical Sanger sequencing was available. Selecting target genomic regions to serve as proxy for the full-length genome is a popular approach for studies of fungal diversity, as it is in the determination of bacterial diversity in microbiome studies. The fungal ribosomal RNA gene cluster (rRNA) is the region most commonly selected as proxy, with sequencing efforts primarily targeting the 18S small subunit rDNA (SSU), 28S large subunit rDNA (LSU) or the internal transcribed spacer (ITS) [20]. Although 28S has gradually been eliminated as a target sequence because of its lack of discrimination power for many species, the debate over whether the 18S or the ITS sequences are most useful is still fierce. Compared with 18S, ITS is more diverse and enables greater genus-level phylogenetic placement. However, in our own experience, the higher conservation of the 18S facilitates the amplification of rDNA from various fungi, and also enables the detection of non-fungal eukaryotes, such as the parasitic protozoa *Leishmania* and *Toxoplasma* [21,22].

Which region within the ITS is selected as a target also remains a matter of choice. Several studies amplify the ITS1 or ITS2 regions, whereas others favor amplifying the entire length of ITS1-5.8S-ITS2 (Table 2; Figure 1). This selection should be made carefully because some primers, such as the ITS1 and ITS1-F primers, are biased toward amplification of *Basidiomycetes*, whereas others, such as the ITS2 and ITS4 primers, are biased toward *Ascomycetes* [23]. The quantitative evaluation and rational design of improved ITS primers are still badly needed, and experience gained in the evaluation of 16S primer sets for bacterial microbiome studies could provide a good model to follow in this endeavor [24]. To judge the accuracy of different primer pairs in taxonomic classification, it would be worth sequencing the full-length ITS, trimming it to simulate the different amplicons that would be obtained by various primer pairs, and

comparing them back with the full-length ITS. Similarly, and as has been done for bacterial species [25], it may be interesting to construct a 'mock' community (MC) with a known composition of fungal species, amplify the rDNA with various primer pairs, and compare the abundance of each species detected with the actual original proportions.

Thus, at present, no common view exists as to the single best fungal rDNA region to select for deep-sequencing analysis. If the goal of the study is to measure the abundance of specific fungi, then using the same set of primers for each mycobiome included in the study is important; but if the intent is to characterize fungal diversity, then a combination of different regions would probably cover more fungal species and thus produce more comprehensive results. Our experience leads us to believe that the efficiency of amplification and the size of the amplicon generated also dictate which portion is the most productive, with shorter amplicons being more consistently generated.

Several NGS platforms that vary in their characteristics are available for mycobiome sequencing. Despite its relatively high cost, pyrosequencing on the Roche/454 GS-FLX is the method most commonly used in mycobiome studies because it achieves the longest sequence reads (500 bp). Other, newer NGS platforms, including Illumina's HiSeq and MiSeq and Life Technologies' Ion Torrent, have also demonstrated their potential recently. Based on results obtained in bacterial microbiome studies, the HiSeq platform provides the highest data output at the lowest cost (50 times less expensive than 454 pyrosequencing), whereas MiSeq is more appropriate when longer read length and quick turn-around time are the priority [26,27]. The Ion Torrent (Ion PGM™ Sequencer and Ion Proton™ Sequencer), with its new protocols leading to 400 bp sequence reads, has also become competitive, providing a low-cost, scalable and high-throughput solution [28].

Analysis of sequence data also presents a number of issues relating to methodology. First, the pipeline must be selected. Two of the most commonly used pipelines in the analysis of microbiome sequencing data are QIIME (<http://qiime.org>) and mothur (<http://www.mothur.org>). Built upon a series of bioinformatic tools, both pipelines allow: the trimming, screening, and alignment of sequences; the assignment of operational taxonomic units (OTUs); phylogenetic analyses; and determination of fungal diversity within and across groups (referred to as α and β diversities) [29,30]. In addition, pipelines that are specific for mycobiome studies, such as CloVR-ITS and BROCC, have been developed recently [21,31]. A database against which the amplified sequences can be compared must also be selected, but a database as rich as that for bacterial 16S rDNA is still lacking for fungi, as is the capability to categorize fungal rRNA sequences at the

Table 1. Summary of culture-independent methods for studying the mycobiome

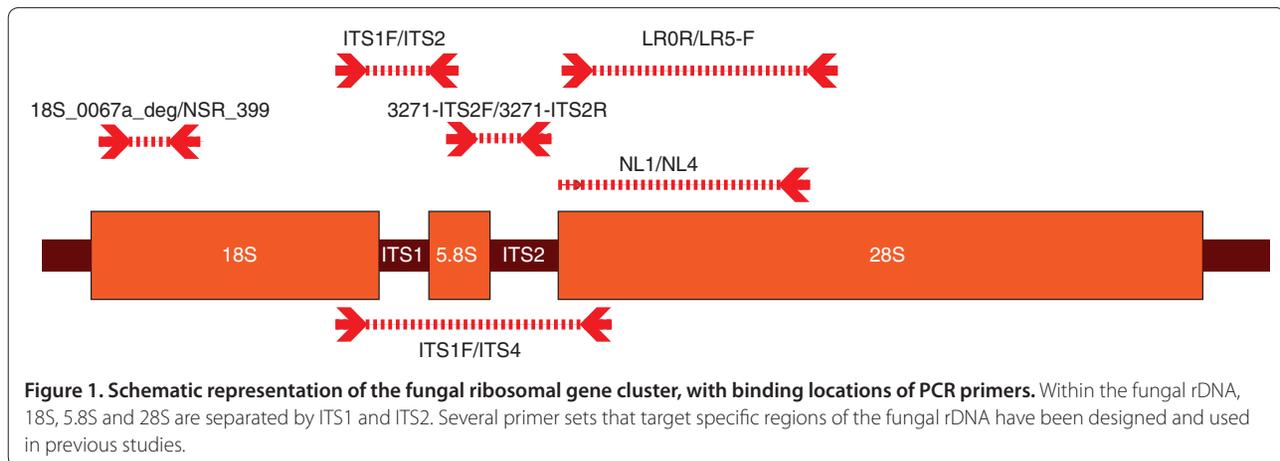
Method	Procedure	Strength	Weakness
RFLP [10,60,61]	<ol style="list-style-type: none"> 1. PCR of rDNA 2. Build the clone library 3. Digest with endonucleases 4. Run capillary electrophoresis 		
OFRG [62]	<ol style="list-style-type: none"> 1. PCR of rDNA 2. Build the clone library 3. Hybridize with oligonucleotide probes 	Allow comparisons of fungal abundance across groups	<ol style="list-style-type: none"> 1. Considerable intraspecific variability 2. Not specific enough to differentiate fungi at the level of species
DGGE [14]	<ol style="list-style-type: none"> 1. PCR of rDNA 2. Build the clone library 3. Run the denaturing gel electrophoresis 4. Analyze the patterns of the bands 		<ol style="list-style-type: none"> 3. Unable to quantify the proportion of each type of fungi in the mycobiome
<i>In situ</i> hybridization [14]	<ol style="list-style-type: none"> 1. Process biopsy sample 2. Probe hybridization 		
Sanger sequencing [50]	<ol style="list-style-type: none"> 1. PCR of rDNA 2. Build the clone library 3. Sanger sequencing 	Specific enough to differentiate between species	High cost [63]
Pyrosequencing [18,64]	<ol style="list-style-type: none"> 1. PCR of rDNA 2. Pyrosequencing 		<ol style="list-style-type: none"> 1. Homo-polymerization 2. Environmental contamination

Table 2. Summary of primers for fungal rDNA amplification used in mycobiome studies

Amplicon	Primer sets	Primer sequence	Length*	Citation
ITS	ITS1	ITS1F/ITS2	260 bp	[8,31]
	ITS2	3271-ITS2F/3271-ITS2R	340-360 bp	[19]
	ITS1-5.8S-ITS2	ITS1F/ITS4	550 bp	[65]
LSU	LROR_F/LR5-F	CCGCTGAACTTAAGCATATCAATA CGATCGATTGACGTCAGA	860 bp	[65]
	NL1/NL4	GCATATCAATAAGCGGAGGAAAAG GGTCCGTGTTCAAGACGG	600 bp	[66]
SSU	18S_0067a_deg/NSR 399	AAGCCATGCATGYCTAAGTATMA TCTCAGGCTCCYTCCGG	350 bp	[21]

level of subspecies. Research groups currently use the fungal rDNA databases of UNITE (<http://unite.ut.ee/>), which includes 6,816 ITS sequences from 1,977 species within 418 genera of fungi [32,33], and SILVA (<http://www.arb-silva.de/>), which includes 6,571 18S and 1,753 28S sequences from fungi in its release 111. Useful as these databases are, they do have some limitations that affect taxonomic assignments. For example, many synonyms and misclassifications are found in the fungal nomenclature; and sexual and asexual forms of a fungal species can be classified as different taxa [21]. Recently,

Findley and colleagues optimized the current ITS database by fixing many of the inconsistencies described in taxonomic entries [22]. They also implemented a species-level resolution to skin-associated *Malassezia* within the software pplacer [34], which provides phylogenetic placement of the sequences. Despite these advancements, we still need to improve the reliability of fungal analyses by pursuing a more systematic evaluation of current databases to determine whether the mycobiomes analyzed to date are indeed well characterized.



Composition and role of the mycobiome in health and disease

The ultimate aim of human mycobiome studies is to uncover the role that fungal populations play in affecting health. Unfortunately, owing to the limitation of culture-dependent methods in mycology, most early studies were restricted to the analysis of a few species: *Candida albicans*, *Candida glabrata*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and the dimorphic fungi (*Coccidioides*, *Histoplasma* and *Blastomyces*) [9]. The development of culture-independent methods has expanded our knowledge of the mycobiomes found in different body sites and their association with disease (Table 3). For example, specific organs that were previously thought to be sterile, such as the lung [19], are now known to harbor a variety of fungi. In other body sites previously known to be colonized with fungus, the fungal variety detected by classical methods is much less than that discovered by newly developed methods. Figure 2 shows an integrated analysis of different mycobiomes reported in the literature. Fungal distributions show significant differences among distant body sites, whereas similar patterns of distribution were found in mycobiomes from nearby sites. For example, *Cladosporium* species, *Aspergillus* species and *Penicillium* species all dominate other fungal genera in both in both oral and nasal cavities [8,35].

Although the association of many mycobiomes with various diseases has been reported, more associations will undoubtedly be characterized in the future. For example, immune-suppressed (for example, HIV-positive) individuals are more likely to contract opportunistic fungal (and protozoa) infections than are healthy persons; thus, mycobiome studies on immune-suppressed individuals are needed to uncover more relevant fungal species, and possibly to identify the mechanistic link between fungal pathogenesis and immune suppression. In lung disease, several studies have attempted to characterize the mycobiome in cystic fibrosis (CF) [11,12,19].

These studies suggest that the fungal diversity is lower in CF patients than in healthy people. The lung mycobiome has not yet been determined in those with chronic obstructive pulmonary disease (COPD) or asthma, although it may have an effect on the progression of these conditions [18].

The mycobiomes present in different body sites potentially interact with each other. At present, the gastrointestinal-respiratory interaction is understood most clearly: a disturbance of the gut mycobiome by *C. albicans* impacts allergic pulmonary disease induced by *A. fumigatus* in the lung mycobiome [36]. Translocation into the bloodstream and subsequent circulation of molecules from fungi, such as RNA, DNA or peptidoglycans, may initiate systemic immune responses and lead to disease remote from the initial site of fungal infection [37,38].

Interactions between the mycobiome and the bacterial microbiome may also play a role in health and disease (Table 4). In some cases, the occurrence of bacteria correlates positively with the presence of fungi; for example, *Mycobacterium* superinfection sometimes occurs along with aspergillosis [39]. In other cases, bacteria compete with fungi; the growth of *Candida* species and possibly other fungi is suppressed when *Pseudomonas aeruginosa* dominates in CF [40]. Various hypotheses have been proposed to explain the dual nature of this interaction. In the case of *Mycobacterium* and aspergillosis, the commensalism of bacteria and fungi may synergistically strengthen their resistance to environmental pressure, such as antimicrobial agents [41]. In the case of *Candida* and *Pseudomonas*, the inhibition of fungal growth by the bacteria may better meet the nutritional requirements of the bacteria, allowing them to secure more attachment sites on the host cell [12]. Regardless of whether the interaction between a mycobiome and a bacterial microbiome is synergistic or competitive, it has the potential to alter both the intrinsic

Table 3. Summary of mycobiomes in different body sites

Sequencing method/ specimen type (sample size (n))	Health status or disease	Fungal composition of the mycobiome	Citation
Oral cavity			
Pyrosequencing/oral rinse samples (n = 20)	Healthy	<i>Candida</i> (22.2%), <i>Cladosporium</i> (19.4%), <i>Aspergillus</i> (11.1%), <i>Fusarium</i> (5.6%), <i>Glomus</i> (5.6%), <i>Penicillium</i> (4.2%), <i>Alternaria</i> (4.2%), <i>Saccharomycetales</i> (13.9%), <i>Cryptococcus</i> (2.8%), <i>Ophiosoma</i> (2.8%), <i>Phoma</i> (2.8%), <i>Schizosaccharomyces</i> (2.8%), <i>Zygosaccharomyces</i> (2.8%)	[8]
Culture and morphologic observation/oral mucosal swabs (n = 30)	AIDS	<i>Candida</i> (100%)	[67]
Nasal cavity			
Culture and morphologic observation/nasal mucosal swabs (n = 48)	Allergies	<i>Cladosporium</i> (29.4%), <i>Penicillium</i> (20.6%), <i>Alternaria</i> (11.7%), <i>Aspergillus</i> (11.7%), <i>Rhodotorula</i> (2.9%), <i>Chrysonilia</i> (2.9%), <i>Paecilomyces</i> (5.8%), <i>Stemphylium</i> (2.9%), uncultivated <i>Ascomycota</i> (2.9%), <i>Cladophialophora</i> (2.9%), others (2.9%)	[35]
Lung			
Pyrosequencing/sputum samples (n = 83)	Cystic fibrosis	<i>Candida</i> , <i>Saccharomyces</i> , <i>Malassezia</i> , <i>Fuscoporia</i> , <i>Fusarium</i> , <i>Acremonium</i> , <i>Thanatephorus</i> , <i>Cladosporium</i>	[12]
Pyrosequencing/sputum samples (n = 4)		<i>Candida</i> (74.98%), <i>Neosartorya</i> (16.68%), <i>Malassezia</i> (2.95%), <i>Hyphodontia</i> (1.04%), <i>Kluyveromyces</i> (1.02%), <i>Aspergillus</i> (0.93%), <i>Penicillium</i> (0.70%), <i>Peniophora</i> (0.43%), <i>Clavispora</i> (0.29%), <i>Piptoporus</i> (0.28%), <i>Dioszegia</i> (0.27%), <i>Phlebiopsis</i> (0.09%), <i>Stereum</i> (0.07%), <i>Torulasporea</i> (0.04%), <i>Chalara</i> (0.04%), <i>Physalospora</i> (0.04%), <i>Eurotium</i> (0.03%), <i>Cryptococcus</i> (0.03%), <i>Quambalaria</i> (0.03%), <i>Nectria</i> (0.03%), <i>Didymella</i> (0.02%), <i>Saccharomyces</i> (0.02%), <i>Sporobolomyces</i> (0.03%), <i>Phaeosphaeria</i> (0.01%), <i>Strobilurus</i> (0.01%)	[19]
DGGE/sputum samples (n = 5)		<i>Candida dubliniensis</i> , <i>C. albicans</i> , <i>C. parapsilosis</i> , <i>Aspergillus fumigatus</i>	[11]
Gut			
RFLP/fecal samples (n = 106)	Hepatitis B cirrhosis and chronic hepatitis B	<i>Candida</i> (33.78%), uncultured fungi (12.53%), <i>Aspergillus</i> (7.99%), <i>Simplicillium</i> (5.65%), <i>Chaetomium</i> (2.46%), <i>Galactomyces</i> (2.33%), <i>Rhizopus</i> (1.96%), <i>Walleimia</i> (1.10%), <i>Fusarium</i> (1.10%), <i>Iodophanus</i> (0.12%), <i>Penicillium</i> (0.49%), <i>Saccharomyces</i> (25.18%), uncultured <i>Pezizomycotina</i> (0.86%), uncultured <i>Pucciniomycotina</i> (1.10%), uncultured <i>Agaricomycotina</i> (0.74%), <i>Aureobasidium</i> (0.61%), <i>Hyphozyma</i> (0.49%), <i>Asterotremella</i> (0.49%), <i>Cryptococcus</i> (0.49%), <i>Doratomyces</i> (0.37%), <i>Ophiocordyceps</i> (0.12%)	[10]
Pyrosequencing/fecal samples (n = 10)	Healthy	<i>Walleimia</i> , <i>Trichocomaceae</i> , <i>Saccharomycetaceae</i> , <i>Rhodotorula</i> , <i>Pleosporaceae</i> , <i>Metschnikowiaceae</i> , <i>Cystoflobasidiaceae</i> , <i>Ascomycota</i> , <i>Amphisphaeriaceae</i> , <i>Agaricaceae</i>	[21]
Sanger sequencing/pouch endoscopic biopsies (n = 57)	Inflammatory bowel disease (including Crohn's disease and ulcerative colitis)	<i>Rhodotorula</i> (16.08%), <i>Galactomyces</i> (0.60%), <i>Trametes</i> (9.52%), <i>Pleospora</i> (7.14%), <i>Sclerotinia</i> (8.34%), <i>Penicillium</i> (7.74%), <i>Bullera</i> (4.17%), <i>Ustilago</i> (4.17%), <i>Candida</i> (2.38%), <i>Chaetomium</i> (2.38%), <i>Flammulina</i> (1.79%), <i>Dacrymyces</i> (1.79%), <i>Exidiopsis</i> (1.19%), <i>Sirococcus</i> (1.19%), uncultured basidiomycete (11.31%), <i>Botryotinia</i> (4.17%), <i>Filobasidium</i> (3.51%), <i>Sporobolomyces</i> (2.98%), uncultured ascomycete (2.38%), uncultured ustilaginomycete (1.79%), <i>Trichosporon</i> (1.19%), <i>Aureobasidium</i> (0.60%), <i>Raciborskiomyces</i> (0.60%), <i>Dothideomycete</i> (0.60%), <i>Cladosporium</i> (0.60%), <i>Madurella</i> (0.60%), <i>Tricholoma</i> (0.60%), <i>Graphiola</i> (0.60%)	[14]
Skin			
RFLP/limb skin swabs (n = 8)	Psoriasis	<i>Malassezia</i> , <i>Paracoccidioides</i> , <i>Blastomyces</i> , <i>Histoplasma</i> , <i>Coccidioides</i> , <i>Microsporum</i> , <i>Trichophyton</i> , <i>Sporothrix</i> , <i>Trichoderma</i> , <i>Hortaea</i> , <i>Anguillospora</i> , <i>Candida</i> , <i>Pneumocystis</i> , <i>Cryptococcus</i> , <i>Trichosporon</i> , <i>Rhodotorula</i> , <i>Rhizopus</i> , <i>Absidia</i>	[68]

Continued overleaf

Table 3. Continued

Sequencing method/ specimen type (sample size (n))	Health status or disease	Fungal composition of the mycobiome	Citation
Sanger sequencing/face skin swabs (n = 9)	Atopic dermatitis	<i>Malassezia</i> (67.9%), <i>Cladosporium</i> (5.5%), <i>Candida</i> (5.2%), <i>Cryptococcus</i> (5.2%), <i>Rhodotorula</i> (4.0%), <i>Trichosporon</i> (2.6%), <i>Meyerozyma</i> (2.0%), <i>Alternaria</i> (1.9%), <i>Debaryomyces</i> (0.3%), <i>Phialophora</i> (0.3%), <i>Aspergillus</i> (0.2%), <i>Davidiella</i> (0.1%), <i>Mrakia</i> (0.1%), <i>Wallemia</i> (0.1%), <i>Sporobolomyces</i> (0.2%), <i>Penicillium</i> (0.2%), <i>Saccharomyces</i> (0.1%), <i>Symptodiomyces</i> (0.1%), <i>Tilletiopsis</i> (0.1%), <i>Apioplagiostoma</i> (0.1%), <i>Toxicocladosporium</i> (1.5%), <i>Wickerhamomyces</i> (0.7%), <i>Aureobasidium</i> (1.9%), <i>Exophiala</i> (0.1%), <i>Gibellulopsis</i> (0.1%), <i>Persiciospora</i> (0.1%), <i>Trametes</i> (0.1%)	[50]
Pyrosequencing/scalp swabs (n = 7)	Dandruff-afflicted scalps	<i>Filobasidium</i> (33.85%), <i>Penicillium</i> (3.44%), <i>Malassezia</i> (1.91%), <i>Eupenicillium</i> (0.04%), <i>Acremonium</i> (57.91%), uncultured soil fungus (2.47%), <i>Cryptococcus</i> (0.26%), <i>Didymella</i> (0.05%), <i>Rhodotorula</i> (0.05%), <i>Coniochaeta</i> (0.00%), uncultured <i>Ascomycete</i> (0.02%)	[66]
Culture and morphologic observation/foot skin swabs (n = 129)	Foot disease in soccer athletes	<i>Trichophyton</i> (76.4%), <i>Candida</i> (20.0%), others (3.6%)	[69]
Vagina			
Pyrosequencing/vaginal mucosal swabs (n = 494)	Healthy	<i>Candida</i> (34%), <i>Pichia</i> (2%), <i>Eurotium</i> (1%), <i>Alternaria</i> (1%), <i>Rhodotorula</i> (1%), <i>Cladosporium</i> (2%), <i>Davidiellaceae</i> (3%), uncultured <i>Saccharomycetales</i> (6%), uncultured <i>Epicoccum</i> (1%), other minorities (49%)	[63]
Conjunctiva			
Culture and morphologic identification/conjunctiva swabs (n = 61)	Leprosy (Hansen's disease)	<i>Candida</i> (26.67%), <i>Aspergillus</i> (13.33%), <i>Geotrichum</i> (6.67%), <i>Acremonium</i> (6.67%), <i>Alternaria</i> (6.67%), <i>Chaetonium</i> (6.67%), <i>Drechslera</i> (6.67%), <i>Penicillium</i> (13.33%), <i>Cladosporium</i> (6.67%), <i>Phialophora</i> (6.67%)	[70]

host immune response to pathogens and the susceptibility of the mycobiome and the bacterial microbiome to medical therapy.

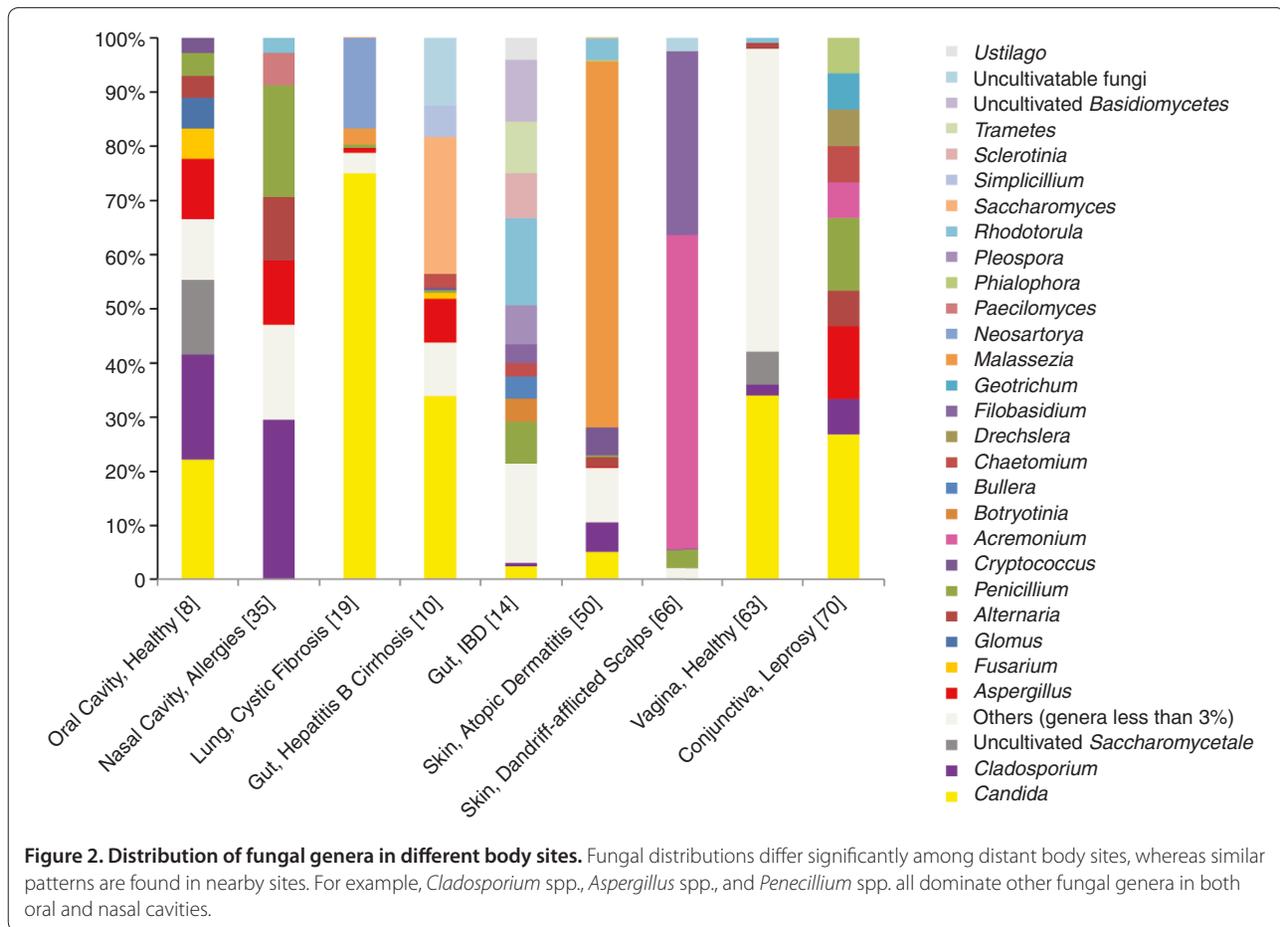
Interactions between the host and the mycobiome are likely to be carefully balanced, leading to clearance, asymptomatic infection, latency, or disease [9]. Several factors in the host will have an effect on mycobiome composition and variations, including host genotype, physiology, immune system, and lifestyle (Figure 3) [42,43]. As some of these factors might change over time, the mycobiome will fluctuate accordingly. The skin mycobiome, for example, is thought to change drastically early in life. Using the genotyping of *Malassezia* species as an indicator, one study demonstrated that it takes 30 days for the skin microbiota to change from its initial state to an adult type [44].

The host immune response influences the composition of the mycobiome (Figure 4). Several pattern-recognition receptors (PRRs) on phagocytes, including TLR-2, TLR-4, dectin-1, dectin-2, and galectin-3, specifically recognize pathogen-associated molecular patterns (PAMPs) of fungi, such as α -mannans, β -mannans, and β -glucans [45-48]. Following this pattern recognition, macrophages and dendritic cells mature and activate T cells through an antigen-presenting process. Depending on which cytokines are stimulated, activated T cells differentiate into either Th-1, which promotes the phagocytosis of fungi, or Th-2, which activates B cells to release fungi-specific

antibodies [16]. Two studies also reported the differentiation of Th-17 following *C. albicans* infection, indicating a potential role for Th-17 in host defense against fungi [47,49]. Despite our depth of knowledge of fungal immunology, it remains to be determined whether these interactions are ubiquitous or tissue specific, and whether some of the interactions mentioned above are due entirely to a pathogenic process or are mainly involved in retaining the homeostasis required for host immune development [7].

Role of the mycobiome: beneficial, commensal or pathogenic?

The pathogenesis of many single fungal species has been well studied, whereas the correlation between the diversity of the whole mycobiome and disease progression is less clearly defined. It is often assumed that fungal diversity should be greater in more severe cases of a disease. For some conditions, such as in inflammatory bowel disease (IBD) [14], atopic dermatitis [50], and hepatitis B cirrhosis or chronic hepatitis B, the diversity of fungi present is proportional to the progression of disease [10]. But in other disease environments, such as the respiratory mycobiome in cystic fibrosis [12,19] and the nasal mycobiome in allergic patients [35], the fungal diversity is inversely proportional to disease progression [35]. To further complicate the issue, the diversity of fungi identified in some diseases has no correlation with



disease status, and the diversity of fungi in samples cluster more according to individuals rather than to health status [51]. Drawing a generalized conclusion about the correlation between diseases and fungal diversity is, thus, difficult. Fungal diversity is more likely to correlate positively with disease status in those with infectious diseases because fungi can exacerbate the infection; in chronic diseases, where fungal infection plays a secondary role in disease pathogenesis, however, an inverse correlation between fungal diversity and disease progression is more likely to occur because the microenvironment (such as that resulting from mucus dysfunction in CF) becomes less suitable for fungal growth.

Like the mycobiome as a whole, individual members of the mycobiome may also play a beneficial or commensal role in the host. Beneficial fungi have been found to be preventive and therapeutic agents, an example being the use of *Saccharomyces boulardii* for the treatment of diarrhoeal diseases [52]. Commensal fungi, such as *Malassezia* spp. and *C. albicans*, usually co-evolve with the adaptive immune system, although in certain cases they may switch from a 'friendly' relationship with the host to a pathological one [53].

Alterations in the mycobiome are frequently reported to be associated with disease progression, but it remains to be elucidated whether this variation is cause or effect. One concern is whether such an alteration in the mycobiome is primary or secondary to an imbalanced bacterial microbiome, as Ott *et al.* [14] proposed for the increase in mycobiome diversity of IBD. A causal relationship could be established if an antimicrobial treatment targeting certain fungal groups were to lead to either exacerbation of disease or cure; on the other hand, if the treatment of the disease were to lead concurrently to modulation of the mycobiome, then it would seem more likely that the mycobiome is being affected by the disease status [54]. Specific mycobiome patterns may be useful as diagnostic or prognostic markers of diseases.

Outlook

Targeting vital fungal species that are associated with disease progression may impact disease severity. Evidence exists to indicate that altering the mycobiome with anti-fungal drugs can improve certain conditions, such as gastrointestinal graft-versus-host disease (GI-GVHD) [49]. Furthermore, specifically controlling the growth of

Table 4. Interaction between the mycobiome and bacterial microbiome

Mycobiome	Bacterial microbiome	Positive or negative correlation	Body sites; disease	Citation
<i>Aspergillus</i> spp.	<i>Mycobacterium</i> spp.	Positive	Lung; aspergillosis	[39]
<i>Blastomyces</i> spp.				
<i>Candida</i> spp.	<i>Staphylococcus epidermidis</i> <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus</i> spp.	Positive	Not applicable; implant- and catheter-related infections	[71]
	<i>Fusobacterium nucleatum</i> <i>Fusobacterium periodontium</i> <i>Fusobacterium sulci</i> <i>Streptococcus gordonii</i> <i>Streptococcus mutans</i>	Positive	Oral cavity; healthy	[72-74]
	<i>Lactobacillus acidophilus</i> <i>Lactobacillus casei</i>	Negative	Intestine; systematic candidiasis	[75]
	<i>Lactobacillus</i> spp.	Negative	Female reproductive tract; fungal vulvovaginitis	[76]
<i>Candida</i> spp.	<i>Pseudomonas aeruginosa</i>	Negative	Lung; CF	[40]
<i>Torulopsis glabrata</i>	<i>Pseudomonas cepacia</i>			
<i>Saccharomyces cerevisiae</i>				
<i>Aspergillus fumigatus</i>				

less desirable fungal species, or controlling biofilm-associated infections, may also be useful in manipulating the complex microflora in diseased body sites [19]. Another viable alternative to direct antimicrobial treatment is to introduce prebiotic and probiotic therapy to restore bacterial commensals. A clinical trial of VSL#3 in pouchitis patients showed that the bacterial diversity in the gut was increased while the fungal diversity was reduced with the use of a probiotic therapy. This effect could result from the restoration of the integrity of a 'protective' intestinal mucosa-related microbiota [13].

With a better understanding of the mechanisms of recognition and modulation in the immune response to fungi, it might become practical to administer immune therapy to treat mycobiome-associated diseases. Effective monoclonal antibodies, which promote opsonization in phagocytosis, activate the complement system or act directly on fungal cells [55], have already been developed for several fungal species, such as *Cryptococcus neoformans* [56], *C. albicans* [57], *Histoplasma capsulatum* [58] and *A. fumigatus* [59]. Fungal-targeted vaccination is another alternative, in both prophylaxis and therapeutics. Fungi that induce a long-term immunity are considered better candidates for vaccination [55], and a few vaccines have already been put forward for clinical trials, such as the killed spherule vaccine against coccidioidomycosis [53].

The limitation of today's immune therapies is that most target only a single fungal species, ignoring the overall mycobiome composition. To date, our knowledge of the mycobiome suggests that interactions among fungi within an environment and between mycobiomes found in different body sites may play an important role in pathogenesis, and that the development of a broad-spectrum monoclonal antibody or a universal vaccine targeting multiple pathogenic fungi would be more promising than therapies targeting a single fungal species. Given that many fungi share a series of PAMPs, it should be feasible to develop a universal immune therapeutic tool that targets such a widely used fungal signature.

Along with the heterogeneity of the mycobiome, genetic polymorphism across human populations also raises different risks for mycobiome-associated diseases (summarized by Romani [53]). For instance, the dectin-1 single nucleotide polymorphism (SNP) Y238X mutation could increase the likelihood of *Candida* colonization and indirectly associate with GVHD [49]. With these recent findings, the significance of sequencing the genomes of hosts with or without disease has been highlighted, and personalized treatment is now often touted as the way forward. Potentially, by classifying people by known genetic markers, we will be able to stratify

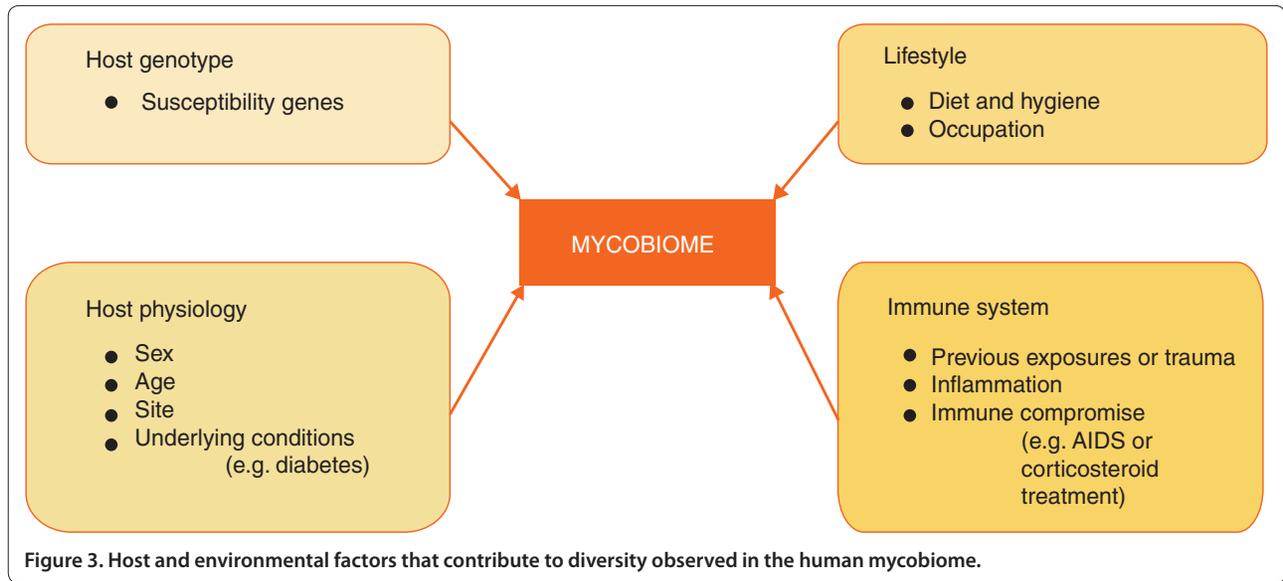


Figure 3. Host and environmental factors that contribute to diversity observed in the human mycobiome.

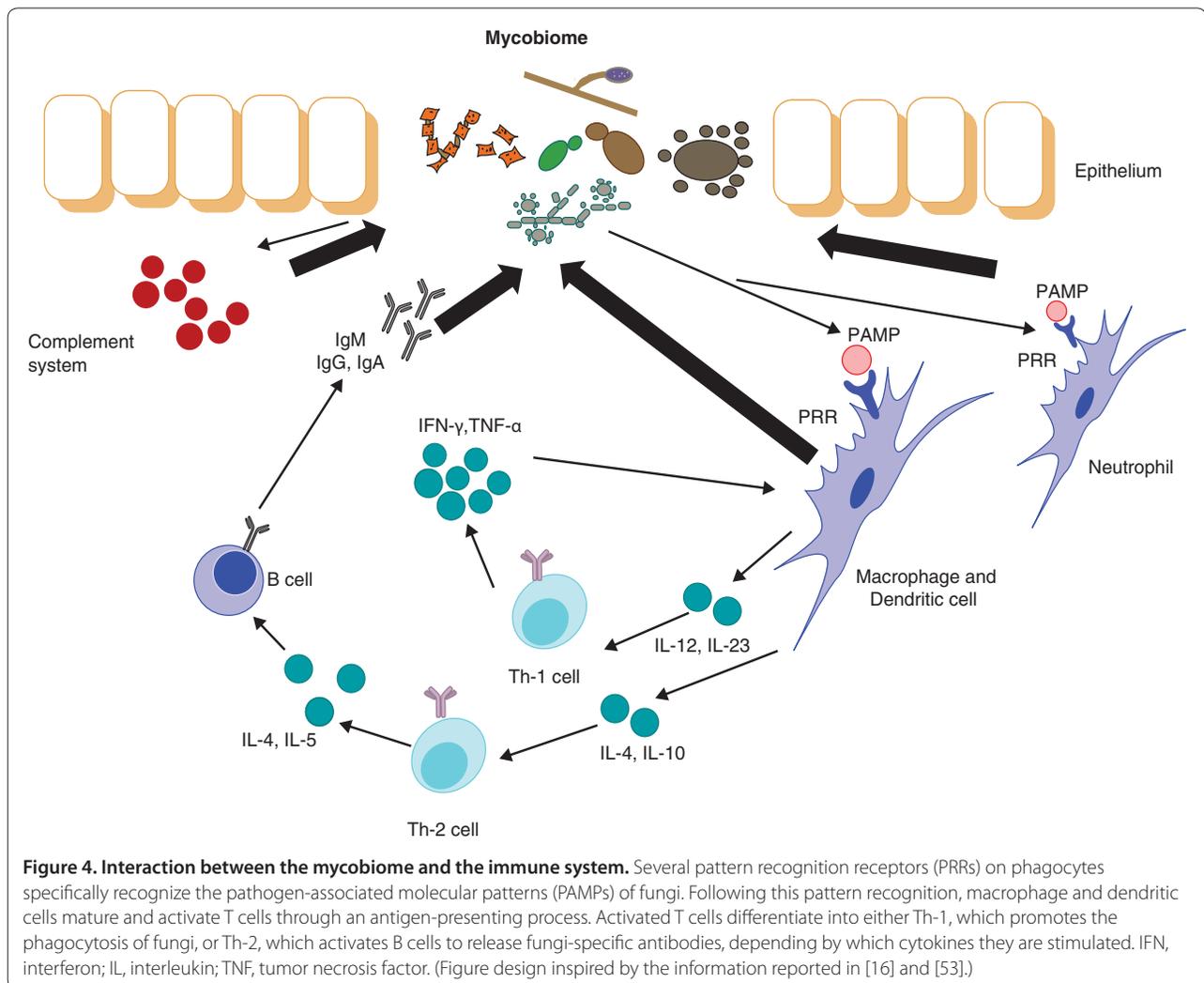


Figure 4. Interaction between the mycobiome and the immune system. Several pattern recognition receptors (PRRs) on phagocytes specifically recognize the pathogen-associated molecular patterns (PAMPs) of fungi. Following this pattern recognition, macrophage and dendritic cells mature and activate T cells through an antigen-presenting process. Activated T cells differentiate into either Th-1, which promotes the phagocytosis of fungi, or Th-2, which activates B cells to release fungi-specific antibodies, depending by which cytokines they are stimulated. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. (Figure design inspired by the information reported in [16] and [53].)

patients with a high susceptibility to fungal infection, and those who would benefit from antifungal agents, thereby optimizing the therapeutic effect and reducing the risk of antifungal resistance.

Conclusions

Defining the mycobiome has broadened the scope of human microbiome studies. Several mycobiomes in different body sites have been characterized, and diverse mycobiome patterns associated with various diseases. After summarizing the methods used in mycobiome studies and analyzing the role of the mycobiome in health and disease, we propose that combining fungal characterization with a generalized assessment of the microbiome will expand our understanding of the microbial environment in disease progression. The mycobiome contributes to disease through the interaction between different biomes as well as through the interaction between the mycobiome and the host. Future studies characterizing the mycobiome will be instrumental in understanding disease pathogenesis and in developing novel therapies.

Competing interests

The authors declare that they have no competing interests.

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Abbreviations

CF, cystic fibrosis; DGGE, denaturing gradient gel electrophoresis; GVHD, graft-versus-host disease; ITS, internal transcribed spacer; LSU, 28S large subunit rDNA; NGS, next-generation sequencing; PAMP, pathogen associated molecular pattern; RFLP, restriction fragment length polymorphism; rRNA, ribosomal RNA gene cluster; SSU, 18S small subunit rDNA.

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