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The emerging world of the fungal microbiome

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Abstract

The study of the fungal microbiota (‘mycobiome’) is a new and rapidly emerging field that lags behind our understanding of the bacterial microbiome. Every human has fungi as part of their microbiota, but the total number of fungal cells is orders of magnitude smaller than that of the bacterial microbiota. However, the impact of the mycobiome on human health is significant, especially as a reservoir for blooms of pathogenic microbes when the host is compromised and as a potential cofactor in inflammatory diseases and metabolic disorders.

Keywords

fungi; microbiome; health; disease; yeast; mucosal

The human microbiome

Advances in high-throughput sequencing and bioinformatics are just beginning to reveal the breadth, depth, and diversity of the fungal world in humans and throughout the environment. Nobody is fungus-free. Although there is currently no reliable estimate of the fungal load in the human body, the total number of fungal cells in the microbiome (the ‘mycobiome’) is orders of magnitude smaller than that of the bacterial microbiome (which is estimated to be about 100 trillion cells) [1]. Every individual’s microbiome contains thousands of different species of microbes, of which 99.9% of the total number of microbial cells belong to only a few species. The less abundant (<0.1%), but more diverse, component of the microbiome has been termed the ‘rare biosphere’ [2,3]. The impact of this rare biosphere on human health is significant because it can act as a reservoir for blooms of pathogenic microbes when the host is compromised. A recently described example of a low abundance species of the bacterial microbiome altering local microbiome composition, host immune responses, and driving development of inflammatory disease has been reported for *Porphyromonas gingivalis* in the development of periodontitis [4]. Humans have a fungal microbiome and it is clearly part of the rare biosphere of their entire microbiome (Figure 1). The vast majority of studies have focused on fungal outgrowth when the host is compromised, with little known about the dynamics of the mycobiome during health. This is a new and rapidly emerging field that lags behind our understanding of the bacterial microbiome. This review will discuss what is known about some of the members of the mycobiome, highlight recent culture-independent studies, and discuss implications of changes in the mycobiome as a potential cofactor in inflammatory diseases and metabolic disorders.

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The fungal microbiome

Disease caused by indigenous fungal organisms such as *Candida albicans* in immunocompromised and antibiotic-treated individuals is a major cause of morbidity and mortality in critical care settings, as well as playing a major role in other diseases of the mouth, stomach, and vaginal tract. Some disease-causing fungi, such as *Histoplasma, Coccidiodes, Paracoccidioides, Blastomyces*, and *Aspergillus*, are considered to be of exogenous origin and, therefore, considered strict pathogens. Others, such as *Candida* and *Malassezia* can be found at significant levels on mucosal surfaces and the skin, even in healthy hosts, and it is from within their niche in the microbiome that they can cause disease. *Cryptococcus neoformans* can exist at low levels for decades in the lungs without causing disease but then bloom to cause a life-threatening illness during immunosuppression. *Pneumocystis* is an example of a fungus exhibiting species-specific growth and can cause pneumonia in immunocompromised individuals, most likely as an outgrowth from an indigenous niche because it can be found at low levels in healthy individuals. Thus, these latter four types of fungi are potential pathogens, that is, commensals when the host environment is healthy or undisturbed, and pathogenic when the host environment is compromised or disturbed (Figure 2).

Unlike for some members of the bacterial microbiota, there is no strong evidence for a mutualistic or beneficial relationship with the fungal microbiome. However, there is also little research in this arena. One example of a beneficial fungus in man is *Saccharomyces cerevisiae var. boulardii*, a well-described probiotic for the relief of gastroenteritis in animal systems and some humans [5]. *S. boulardii* can be considered a beneficial yeast, although it can cause fungemia in humans when it colonizes indwelling catheters from aerosolization during opening of lyophilized packets [6]. Because many host–microbe mechanisms are generally conserved between human–fungi and plant–fungi interactions, this suggests that there still remains much to be discovered about the spectrum of the ecological relationships between the fungal microbiome and its host. The roles of the fungal microbiome as a cofactor in inflammatory and metabolic disorders and in modulating the bacterial microbiome (thereby indirectly impacting the host) are areas of emerging interest in human health.

Detection of fungi

The methods and approaches to detect, identify, and analyze fungal communities on mucosal surfaces have significantly evolved over the last decade. Historically, the ability to culture fungi was critical for their detection and identification: via microscopy, biochemical assays, and selective media. With the introduction of polymerase chain reaction (PCR) technology to the field in the early 1990s, DNA-based detection began to revolutionize the methodologies for detection of fungi. As genomic sequencing was applied to both environmental and medically important fungi, the database for studying fungi began to expand. Although the fungal databases lag way behind those for bacteria, such as the Ribosomal Database Project, it is now clear that there are many fungi that elude detection by standard culture techniques. The major target for culture-independent analyses and database generation has been the genetic locus containing the 18S, 5.8S, and 28S rRNA genes as well as the internal transcribed spacer regions (ITS1 and ITS2), the DNA that encodes for the nonfunctional RNA transcribed during rRNA synthesis (Figure 3). Next generation sequencing (specifically the use of pyrosequencing of the fungal ITS1 and ITS2 regions) and clone libraries (built from the 18S, 5.8S, 28S regions as well as ITS1 and ITS2) have revealed a previously unrecognized diversity of fungi in the microbiome, both culturable and nonculturable, as members of the rare biosphere [7–11]. The role of the diverse fungal biosphere in host metabolism, immunity, and modulation of the bacterial microbiome

remains largely unknown. However, the surprising identification of organisms such as *Cryptococcus* in 20% of oral samples of healthy individuals [10] raises the possibility that even culturable fungi may exist at low levels in a difficult-to-culture state in healthy individuals, thereby providing a niche for outgrowth and disease when host defenses and/or competitive exclusion become compromised.

Fungal microbiome of mucosal sites

Chronic colonization of mucosal surfaces, including the urogenital tract, oral cavity, and gastrointestinal (GI) tract with fungi is common, especially for *Candida* spp., and carriage rates of *Candida* among healthy adults ranges from 30 to 70% [12,13]. The genus *Candida* includes approximately 160 species, most of which are adapted to live in mammalian hosts, causing infections in both immunocompetent and immunocompromised individuals [14]. In humans, *C. albicans* is the most abundant and significant species associated with disease. Other medically important species include *Candida glabrata, Candida rugosa, Candida parapsilosis, Candida tropicalis, Candida dubliniensis, Candida krusei*, and *Candida lusitaniae. Candida* spp. are uniquely adapted to their human hosts and acquisition occurs at birth or with later human contact. Strain types are often the same in the same individual at different tissue sites. In addition, the population of strains associated with infection is statistically the same as that isolated in the absence of infection [15]. Because *Candida* can colonize a variety of microbiota-containing host niches (both biotic tissues and abiotic indwelling medical devices), its pathogenic potential from within the microbiome is related to its ability to adapt, survive, and grow in constantly changing environments. These include the potential to adhere to and invade host cells, the ability to undergo morphological transitions and form biofilms, and specific metabolic and fitness traits. These latter traits could either allow *Candida* to synergize with or antagonize other members of the microbiota; likewise, these traits may facilitate asymptomatic colonization or symptomatic infection in the face of host immune defenses [16].

Most individuals are asymptomatically colonized with this organism. However, when environmental conditions permit the outgrowth of *C. albicans*, colonization can lead to infection and invasion of host tissues. *Candida* infections may remain localized to mucosal sites (e.g., oral or vaginal candidiasis) or may spread hematogenously leading to candidemia or deep-seated mycoses of other tissues, which can lead to death. Two environmental variables that can lead to increased levels of *C. albicans* in the host are immunosuppression and antibiotic treatment, the latter of which decreases bacterial colonization resistance against *Candida* (Figure 2). The risk factors for mucosal overgrowth of *Candida* and/or inflammation are niche specific [17]. For example, immunosuppression is associated with oropharyngeal *Candida* disease, but not vaginal candidiasis, whereas antibiotic use leading to *Candida* outgrowth and host sensitization can lead to the transition from asymptomatic carriage to chronic vaginal inflammation, that is, ‘yeast infection’ [18].

Although carriage is usually asymptomatic, anti-*Candida* antibodies are often detected in normal individuals, indicating the elaboration of adaptive immune responses to commensal *Candida* [19]. It is unclear how *C. albicans* remains at mucosal surfaces in the face of adaptive immunity. However, because *Candida* can produce immunomodulatory compounds such as oxylipins, it is tempting to speculate that this commensal organism modulates immune responses to favor its persistence [20]. Modulation of host responses during asymptomatic colonization may in itself lead to deleterious consequences.

Several *C. albicans* factors have been implicated in mediated interactions with the bacterial microbiota. Members of the agglutinin-like sequence family of adhesins mediate *C. albicans* aggregation with other bacteria and yeasts [21]. Another factor that may influence *C.*

albicans interactions with bacteria is farnesol, a quorum sensing molecule. Farnesol represses hyphal growth and early biofilm formation, is required for virulence during disseminated infection and has similar effects on other bacterial pathogens, inhibiting biofilm growth and formation (reviewed in [22]). This may be a strategy used by *C. albicans* growing in biofilms to disperse under conditions of stress, which may also lead to dispersal of bacteria in the polymicrobial biofilm. In addition to cross-kingdom adhesion and quorum sensing, metabolic changes in the environment (pH, metabolic substrate production, inhibitor production, nutrient sensing/sequestering, etc.) and indirect activity on the host response are mechanisms by which *Candida* and other fungi interact with bacteria in the microbiome to regulate fungal levels and host responses to fungal colonization [23].

Fungal microbiome of the skin

The diversity of the microbiome of human skin is an area of intense investigation because changes in the microbiome may be associated with the development or chronicity of many dermatologic conditions or diseases [24]. Cultivation techniques suggested that the fungal microbiome of healthy skin was limited to few genera, mainly *Malassezia* and *Candida. C. albicans* can be carried on the skin, with carriage rates ranging from 50 to 65% among hospital workers [25]. Skin sensitization to *C. albicans* is also strongly correlated with development of atopic dermatitis (AD) [26]. *Malassezia* are lipophilic yeast, classified in the phylum Basidiomycota, and this genus currently includes 14 species isolated from healthy and diseased human and animal skin [27]. *Malassezia* spp. are associated with a variety of skin diseases including psoriasis, dandruff, AD, or eczema, seborrheic dermatitis, and pityriasis versicolor [27]. Molecular methods have revealed that a diverse fungal microbiome exists on the skin and the composition differs between healthy and diseased sites. Overall, these methods have revealed that the skin harbors a diverse fungal community comprised colonizers such as *Malassezia* that increase in numbers in damaged skin, as well as other less numerous colonizers and other transient passengers derived from the environment.

Psoriasis can be triggered and/or exacerbated by a variety of environmental factors, including bacterial and fungal members of the skin microbiome [28]. In studies by Blaser and colleagues, the fungal microbiota of healthy skin and psoriatic lesions was studied through clone library construction and sequencing from different skin sites, using a set of broad-range 18S ribosomal DNA (rDNA) primers and another set specifically targeting the 5.8S rDNA/ITS2 of *Malassezia* [29]. On the healthy skin of one subject, >98% of the clones could be grouped into two Basidiomycota phylotypes that were related to but distinct from *Malassezia furfur* while two psoriatic lesion sites from the same subject contained the same phylotypes but at different ratios. The remainder of the study used *Malassezia*-specific 5.8S/ITS2 primers to analyze a large number of samples from over 20 subjects. Five *Malassezia* species and four unknown phylotypes were identified. Most notably, the species distribution was largely subject-specific, but conserved throughout different sites of healthy skin. Overall, this study demonstrated the predominance of *Malassezia* organisms on healthy human skin, host-specific variation in colonization, relative stability over time on healthy skin, and no consistent patterns of *Malassezia* colonization that differentiated psoriatic skin from healthy skin.

Another study examined the changes in the fungal microbiota of the scalp that accompany dandruff [30]. The investigators used pyrosequencing of 26S rRNA gene libraries derived from healthy and dandruff-afflicted scalp samples. While fungi of the Ascomycota dominated in both healthy and dandruff patients, fungi of the Basidiomycota phyla (which includes *Malassezia*) were significantly increased in dandruff-afflicted scalps. Specific genera that were increased in patients with severe dandruff included *Filobasidium*, which

jumped to 94% of the total basiomycetes, and *Malassezia* (5%), which represents a twofold increase over healthy samples. By contrast, healthy scalps were dominated by basiomycete fungi of the genus *Cryptococcus*. Among the ascomycetes, *Acremonium* spp. dominated in both healthy individuals and dandruff patients. However, *Didymella* was significantly increased in healthy samples, whereas *Penicillium* spp. increased in dandruff patients, particularly in severe cases. This study confirms the previously reported association of *Malassezia* spp. with dandruff, but also reveals the potential for *Filobasidium* in this disease.

AD or eczema is a multifactorial allergic disease that is characterized by a variety of symptoms, notably intense itching, edema, and erythema in affected skin sites. The pathophysiology of the disease involves barrier disruption and immune dysregulation, although the cause and effect sequence is debatable. The fungal microbiome of the skin could participate in exacerbating atopic reactions in patients by shedding allergens, secreting compounds that actively modulate host responses, and/or producing enzymes that damage host cells. *Malassezia* spp. have long been associated with AD, and many studies have focused on this genus in disease [27]. The overall skin fungal microbiota of patients with mild, moderate, or severe AD was compared with healthy subjects using rRNA clone library sequencing [31]. *Malassezia* spp. were predominant in all groups; however, the diversity of non-*Malassezia* yeast microbiota was increased in AD patients. Notably, *C. albicans, Cryptococcus diffluans*, and *Cryptococcus liquifaciens* were detected in all AD samples, but were seldom detected in healthy samples. In particular, *C. albicans* was present in only one healthy subject. AD is also more highly associated with sensitization to *C. albicans*, compared with other fungal species [26]. Increased *Candida*-specific IgE also correlates with severity of disease [32]. These findings suggest that *C. albicans* could be an unforeseen contributor to AD pathogenesis and supports a need for further fungal microbiota analysis on a larger scale.

Microbial biofilms formed from microbiota members contribute to the delayed healing of chronic wounds and a survey of clinical specimens taken from chronic wounds revealed 23% were positive for fungal species [33]. The most abundant fungi were yeasts of the genus *Candida*; however, *Malessezia, Curvularia, Aureobasidium, Cladosporium, Ulocladium, Engodontium*, and *Trichtophyton* were also found to be prevalent components of these polymicrobial infections. Quantification of bacteria versus fungi in these chronic wounds demonstrated that fungi contributed to >50% of the microbial burden in the majority of the wounds. In several cases, addition of antifungal drugs to the treatment regimen resulted in gradual healing of the wound site. This clinical survey demonstrates that the incidence of fungal pathogens in wound biofilm infection is more significant than previously reported and may contribute to the extremely recalcitrant nature of many wounds to antibacterial agents.

Fungal microbiome of the mouth and lungs

The human oral microbiome is comprised at least 700 bacterial taxa [34]. These microbial communities are arranged into surface-localized communities that vary considerably in composition according to sites of establishment. Although the mouth clearly harbors fungi, much remains unknown about the oral fungal communities. Ghannoum and colleagues performed the most comprehensive study to date on the diversity of the fungal microbiome at any human body site, analyzing the fungal microbiome of the mouth [10]. In this study, they found that the distribution of fungal species in the mouth varied greatly between different individuals. In about 20% of the study participants, their oral microbiome included at least one of the following four most common genera of pathogenic fungi: *Candida, Aspergillus, Fusarium*, and *Cryptococcus*. The most abundant *Candida* species were *C. albicans* (in 40% of the subjects), *C. parapsilosis* (15%), *C. tropicalis* (15%), *C. khmerensis*

(5%), and *C. metapsilosis* (5%). Also of note in this study, nonculturable fungi represented almost 40% of the fungi identified in the oral cavity of healthy individuals. One of the most interesting aspects of this study was that 60 nonpathogenic fungal genera were identified in subjects that are ubiquitous environmental microbes. The presence of these microbes in the oral cavities of healthy individuals was not necessarily surprising, because they are most likely acquired from food and mouth breathing, but the observation that transient colonization by environmental fungi may occur in the oral cavity (and upper airways) has potential implications for hypersensitivity diseases.

The lungs, previously believed to be sterile when healthy, clearly harbor a low level bacterial microbiome that changes during disease [35]. To date, little is known about the fungal microbiota of the lungs, with the exception of *Pneumocystis* spp. (described below). In a recent study by Charlson *et al.*, the fungal microbiome of the mouth and lungs in select healthy and lung transplant recipients was analyzed by ITS-based pyrosequencing [7]. The fungal distribution in the oral wash of healthy subjects was similar to the previously published study described above [10]. In the lung transplant recipients, the fungal microbiome of the oral cavity was dominated by *Candida*, likely owing to antibiotic and immunosuppressant use [7]. In the bronchoalveolar lavage of healthy volunteers, there was minimal fungal ITS amplification. By contrast, the bronchoalveolar lavage from lung transplant recipients showed a markedly different pattern, with detectable fungi of either *Candida* spp., *Aspergillus* spp., or *Cryptococcus* spp., oftentimes at significant levels. Because all of the transplant recipients had been treated with antibiotics and immunosuppressants, this first study of the lung fungal microbiome supports the notion that host defense, and perhaps some sort of bacterial microbiome-mediated resistance mechanisms, play a major role in keeping fungal colonization extremely low in the lungs.

One notable member of the fungal microbiome in humans and animals are fungi of the *Pneumocystis* genus [36]. This organism was for a long time considered a unicellular protist. However, the past three decades of research on this organism has firmly established it as a fungus of the Ascomycota phylum. This fungus is highly unusual among medically important fungi in that it appears to have co-evolved with its host and lacks a number of key biosynthetic pathways thereby precluding growth outside its host. Thus, *Pneumocystis* derived from humans will not grow in mice and vice versa. The preferred growth niche in the host is the lungs and new molecular surveys are revealing that it is carried at low levels, even in healthy individuals. This fungus can be spread from individual to individual through airborne transmission, but it can also cause pneumonia following overgrowth in immunocompromised hosts. *Pneumocystis* has also been implicated as a cofactor in a number of chronic pulmonary inflammatory diseases. Thus, the fungus *Pneumocystis* appears to exist as a very low level commensal in the lung microbiome when the host is healthy and becomes pathogenic when the host becomes immunocompromised.

Fungal microbiome of the GI tract

Despite its harsh environment, the stomach harbors a microbiome that can include *Lactobacillus, Helicobacter*, and *Candida* spp. [37–40]. The low pH environment of the stomach selects for colonization by microbes with a high degree of acid tolerance, such as *Candida*. Within the stomach, *Lactobacillus* growth can antagonize colonization by *Candida* (Box 1). Some *Candida* species, such as *C. pintolopesii*, can exist in the murine microbiome without inducing inflammation [38]. Erosions and ulcerations of the mucosal surfaces in the stomach and intestinal tract can also favor the growth of select microbial populations, including *C. albicans* [41]. In humans, gastric ulcers associated with *C. albicans* colonization is a well-documented condition, although generally unappreciated in terms of etiologic

agents of gastric ulceration (discussed in [40]). *Candida* colonization of the GI tract of mice can drive allergic sensitization to food antigens by affecting the mucosal barrier [42].

Despite the extensive literature on the bacterial microbiome of the intestinal tract, little is known about the fungal microbiome of the intestinal tract. As discussed earlier in this review, *Candida* spp. can clearly grow in the intestines, coexist with the bacterial microbiome in the intestines, bloom during antibiotic perturbations of the microbiome, and colonize inflamed mucosa of the intestine. But what about other fungal genera? In one study, using mice from both a specific pathogen-free (SPF) source and from a colony with a restricted bacterial flora (RF mice), a diverse and abundant fungal microbiome was detected by molecular techniques [43]. All four major fungal phyla were detected (Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota) and there were clear differences in the species distribution within the fungal microbiome between the two types of mice. Of note in this study was the lack of *Candida* spp. detected, as well as a lack of culture data to compare with the molecular methods. SPF mice routinely lack culturable fungi and even treatment with a broad spectrum antibiotic, such as cefoperazone, rarely results in the culture of fungi from intestinal samples on fungal media (Sabouraud dextrose agar) [40,44,45]. In a recently published study with Dectin-1 knockout and wild type mice, high levels of indigenous fungi were reported in wild type laboratory mice, especially *C. tropicalis* (a more virulent *Candida* species for damaged mucosa), which accounted for 65% of the fungi detected by ITS1–2 amplicon pyrosequencing [46]. The total fungal loads were estimated to be 5–7% of the fecal material by staining and were easily visualized by immunohistology on mucosal surfaces, although no culture data was presented for *C. tropicalis*, which can easily be cultured and identified on Chromagar Candida. Other studies in mice and humans, either healthy or on antibiotics, have not reported anywhere near that level of colonization with *C. troplicalis* or other fungi [1,43,44,47–49]. The metagenomic analysis performed by the MetaHIT group on fecal specimens collected from 124 healthy, overweight, and obese individuals (including some with inflammatory bowel disease) from Denmark and Spain reported that only 0.1% of the genes in fecal material were of eukaryotic or viral origin, which was consistent with previous reports of fungal 18s rDNA signatures accounting for only 0.03% of the fecal microbiota [1,47]. Thus, as can be summarized from all of these studies, the study of the fungal microbiome in the intestinal tract is in its infancy and much remains to be determined.

Concluding remarks and future perspectives

There is ever-increasing evidence of a significant role for the microbiome in immune regulation, chronic inflammatory diseases, metabolism, and other physiologic processes, including recovery from antibiotics (Box 1). The research to date has focused on the bacterial microbiome; however, clinical and experimental studies are beginning to highlight a role for fungi in these processes. Recent studies have demonstrated that the bacterial GI community reassembly after broad spectrum antibiotics can be altered if the fungal community blooms in the GI tract [40,44] and this can impact the development of allergic airway disease [45,50] (Figure 4). Furthermore, patients with severe asthma with fungal sensitization (SAFS) are often sensitized to *C. albicans* and benefit from antifungal therapy [51]. Colonization of mice with *C. albicans* can result in dietary antigen leak from the stomach [42]. Crohn's disease is associated with a microbiome dysbiosis and the development of antibodies against members of the microbiome [52,53]. This includes anti-*Saccharomyces cerevisiae* antibodies (ASCA), which have been shown to be reactive to an *in vivo*-expressed epitope on *Candida* species, as well as baker's yeast [54]. A recent study using mice that are missing the C-type lectin receptor Dectin-1 demonstrated that these mice had an increased susceptibility to chemically induced [dextran sulfate sodium (DSS)] colitis and this was abrogated by fluconazole and could be exacerbated by repeated oral delivery of

C. tropicalis [46]. This was consistent with the report that *C. albicans* could exacerbate DSS-induced colitis [55] but demonstrated that an indigenous *Candida* population could drive disease.

Evidence is clearly beginning to accumulate that the fungal members of the rare biosphere of the microbiome may play a greater role than previously ascribed in regulation of mucosal health, especially during blooms when the bacterial microbiome is disturbed. More research is needed to catalog the mycobiome in health and disease and also to view these communities through the lens of microbial ecology in terms of membership, presence/absence, levels, and cross-kingdom microbial interactions. Additionally, once we know about membership, what about function? The metabolic maps and genomic databases for fungi lag significantly behind those for bacteria. Future studies will begin to unravel the questions raised here about the levels and composition of indigenous fungi (the mycobiome) in the mouth, lungs, skin, and GI tract in health and during disease, and the mechanisms whereby they may contribute to (or protect from) disease.

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Box 1. *Lactobacillus–Candida* antagonism

The indigenous microbiota of the GI tract is effective at preventing fungi, such as *Candida albicans*, from high-level colonization and disease [56–63] and germfree mice, lacking the indigenous microbiota, are highly susceptible to *Candida* colonization [64]. Mouse models of candidiasis demonstrate that disturbance of the microbiota or immunosuppression are necessary to promote *Candida* colonization. During gastric colonization, *Candida* spp. can induce inflammation or exist as noninflammatory commensal organisms [38,65,66]. Although the exact mechanisms still remain to be determined, it has been suggested that lactobacilli are critical [59,60]. Results from *in vitro* studies implicate the bacterial microbiome in blocking yeast adhesion to the epithelium and producing inhibitor substances (such as volatile fatty acids and secondary bile acids) that can reduce *C. albicans* adhesion, hyphal transformation, and invasion [59,60,66–69]. However, *Lactobacillus*– *Candida* antagonism can be a two-way process whereby the presence of *Candida* can prevent the regrowth of *Lactobacillus* after antibiotics [40,44]. The effect may be more widespread than effects on *Lactobacillus* because recent data suggests that yeast, even as numerically inferior microbes in the intestinal microbiome, have the potential to exert marked effects on overall bacterial community reassembly in the intestine after antibiotics without inducing intestinal inflammation [44].

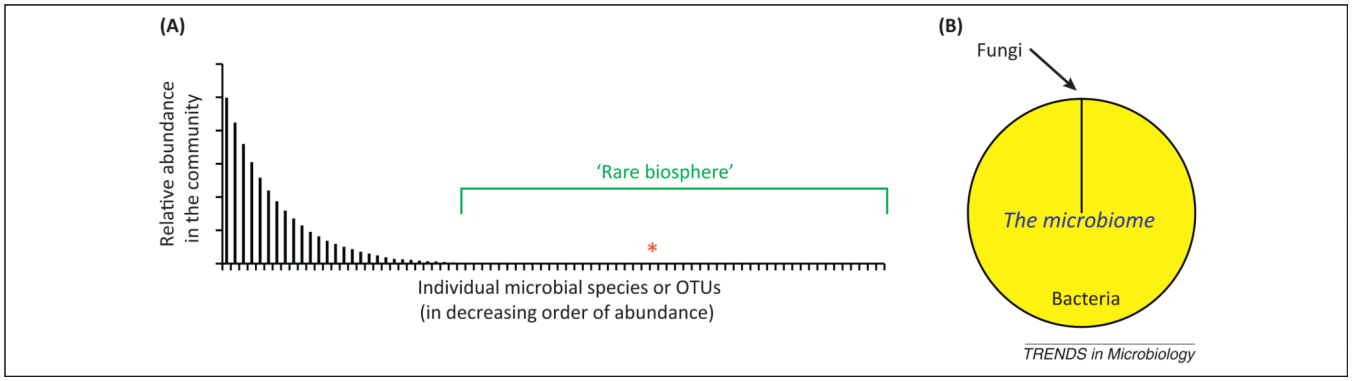

Figure 1.

The rare biosphere and the fungal microbiome. **(A)** Representation of the membership of the microbiome, displayed as a rank abundance curve and illustrating the concept of the 'rare biosphere' within the entirety of the microbiome and the relative position of a microbe at 0.01% abundance (asterisk) in this example. In the complex microbial communities of the mucosa, a relatively small number of species dominate, but hundreds to thousands of low abundance species also exist in the communities. At mucosal sites where bacterial abundance can reach $>10^{10}$/g, a microbe present at 10^4/g would be <0.0001% of the cellular content of the community. Microbial diversity in the microbiome is extensive and the large number of low abundance species (or operational taxonomic units, OTUs) in mucosal samples have been described as part of a rare biosphere [2,3]. However, the rare biosphere serves as a reservoir for potentially pathogenic microbes, such as members of the fungal microbiome, which grow out (bloom) and cause disease when the mucosal environment is disturbed. The rare biosphere may also harbor species that have a disproportionate effect (positive or negative) on the dominant members of the microbiome, a potential pathway by which the fungal microbiome may promote chronic disease. **(B)** Estimated relative cellular abundance of fungi to bacteria in human feces, based upon the MetaHIT metagenomic analysis [1].

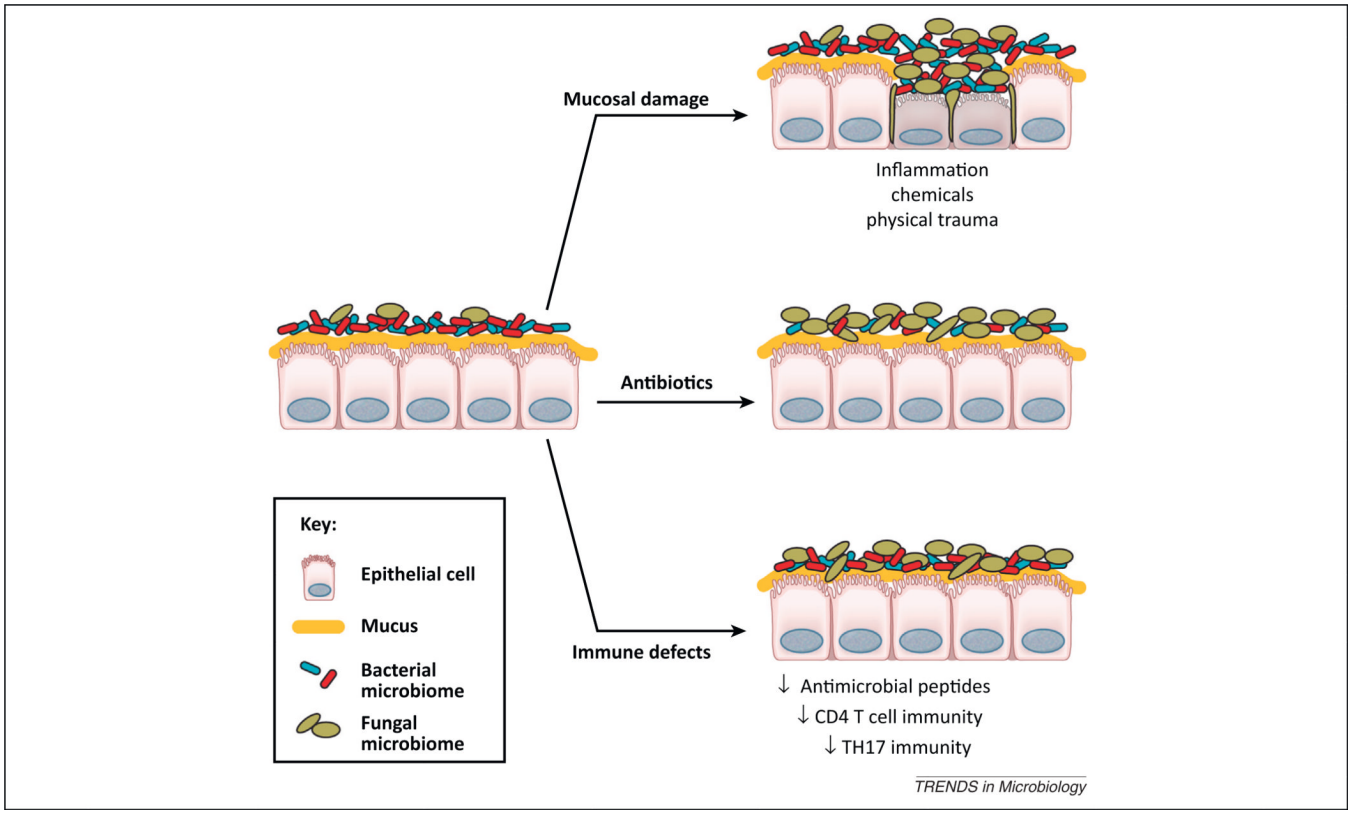

Figure 2.

Mechanisms by which the fungal microbiome can grow out and cause disease on mucosal surfaces. The fungal microbiome exists on mucosal surfaces in a symbiotic relationship with the host and the bacterial microbiome. Any of the following will promote the outgrowth of fungi on a mucosal surface: disruption of bacteria-mediated colonization resistance by antibiotics; damage to the mucosa by uncontrolled inflammatory responses, physical damage, or chemical-mediated injury; or defects in host defenses/immunity that affect innate immunity or specific facets of adaptive immunity.

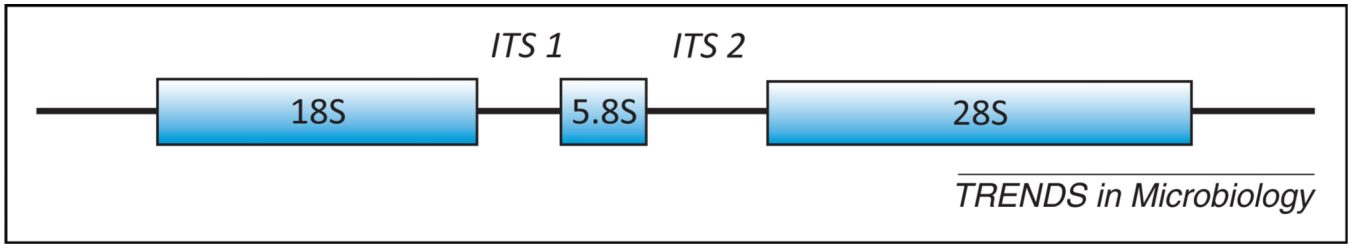

Figure 3.
Fungal ribosomal gene locus. The most commonly used genetic region used for culture-independent molecular analysis of fungal diversity. Both clone library/sanger sequencing and pyrosequencing have been used to map and catalogue this region. High-throughput pyrosequencing has been used for amplicon libraries generated through pan-fungal internal transcribed spacer regions (ITS) primers that span the region between the 3′ end of the 18S gene, includes the entire 5S gene, and ends in the 5′ region of the 28S gene [9]. This region has been previously demonstrated to amplify a wide range of medically relevant fungi by PCR and pyrosequencing [7–11].

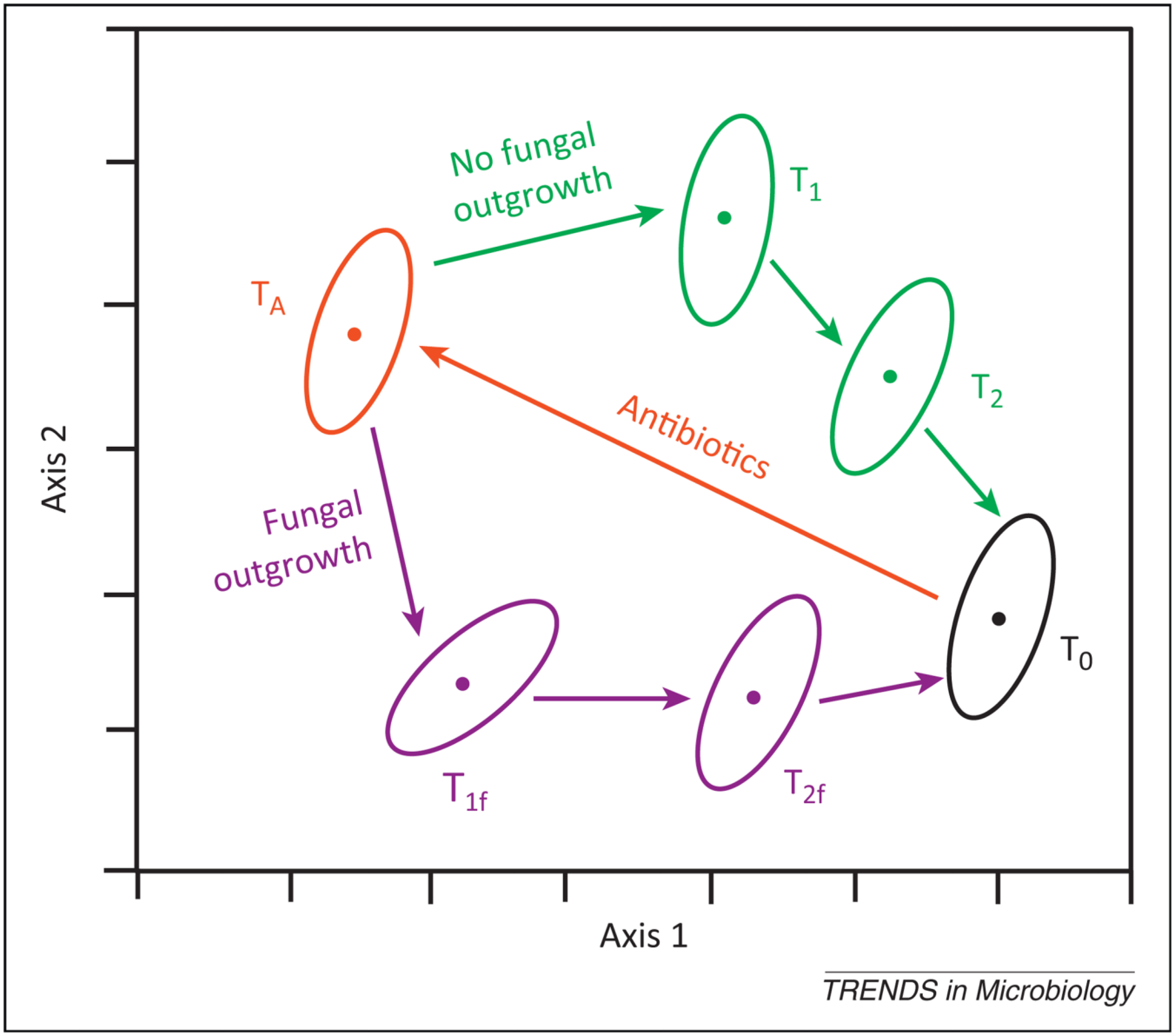

Figure 4.

Hypothetical representation of the effect of the presence and absence of a fungal outgrowth that modulates bacterial community reassembly. Reassembly of the bacterial microbiome after antibiotic therapy is an ordered process ($T_A \rightarrow T_1 \rightarrow T_2 \rightarrow T_0$). Emerging evidence suggests that reassembly may be impacted by the bloom of specific fungal species when colonization resistance is destroyed. This figure provides an illustration of an ecological modeling (ordination) of such a process, in which the bacterial community structure undergoes a different reassembly process ($T_A \rightarrow T_{1f} \rightarrow T_{2f} \rightarrow T_0$) in the presence of a fungal bloom. In this example, the differences in bacterial community structure (membership, richness, and evenness) between the $T_A \rightarrow T_1 \rightarrow T_2 \rightarrow T_0$ path and the $T_A \rightarrow T_{1f} \rightarrow T_{2f} \rightarrow T_0$ path could manifest as secondary changes in microbiome-mediated physiologic processes such as immune regulation or metabolism. Abbreviation: T, timepoint.