Crossing Kingdoms: How the Mycobiota and Fungal-Bacterial Interactions Impact Host Health and Disease

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ABSTRACT

The term “microbiota” invokes images of mucosal surfaces densely populated with bacteria. These surfaces and the luminal compartments they form indeed predominantly harbor bacteria. However, research from this past decade has started to complete the picture by focusing on important but largely neglected constituents of the microbiota: fungi, viruses, and archaea. The community of commensal fungi, also called the mycobiota, interacts with commensal bacteria and the host. It is thus not surprising that changes in the mycobiota have significant impact on host health and are associated with pathological conditions like Inflammatory Bowel Disease (IBD). In this review we will give an overview of why the mycobiota is an important research area and different mycobiota research tools. We will specifically focus on distinguishing transient and actively colonizing fungi of the oral and gut mycobiota and their role in health and disease. In addition to correlative and observational studies, we will discuss mechanistic studies on specific cross-kingdom interactions of fungi, bacteria, and the host.

WHAT IS THE MYCOBIOTA?

Fungi are microeukaryotes that can be found on various mammalian mucosal surfaces, such as the lungs (1-3), the vaginal tract (4, 5), the urinary tract (6, 7), the oral cavity (8-10), and the intestines (9, 11, 12), as well as on the skin (5, 9, 13-15), breast and in breast milk (16) (Fig 1). Historically, research on fungi focused on pathological conditions and fungi as pathogens or pathobionts. For example, expansion of the commensal yeast Malassezia on the skin is associated with a disease known as Pityriasis Versicolor (17). Inhaled spores of the mold Aspergillus fumigatus can germinate and cause invasive lung disease in immunocompromised patients (18, 19). The yeast Candida albicans is arguably the most studied pathogenic fungus and is responsible for a variety of disease conditions, which include vulvovaginal candidiasis in...
women, oropharyngeal candidiasis in infants and immunocompromised patients, and invasive candidiasis with systemic dissemination of *Candida* to peripheral organs (20-23).

Fungi are not only the causative agents of disease but can also be isolated from mammals in the absence of disease (11, 15, 24-26). *C. albicans*, for example, can be frequently isolated as a commensal of the oral cavity, vagina, or gut of healthy individuals and only causes infections if the host immune system is compromised or the local microbiota disturbed (22, 23). However, a culture-dependent approach has a high likelihood to yield an incomplete picture of the total fungal diversity (11, 12). The advent of sequencing technology allowed us to answer important questions about fungi: Do complex commensal communities of fungi exist in or on different mammalian anatomical sites? Which fungi do they comprise? Are they transiently present or do they stably colonize? What are their functions? The last decade has seen a stark increase in publications addressing these questions. We now know that diverse commensal fungal communities exist in and on mammals. These fungal communities are commonly referred to as the fungal microbiota or mycobiota and are the subject of exciting new research.

**METHODS TO ANALYZE THE MYCOBIOTA**

A variety of different methods have been used to detect live fungal cells or fungal genomes. Some of the techniques include direct culturing, enriched culturing, microscopy with fluorescence in situ hybridization (FISH) or immunofluorescence, flow cytometry, amplicon sequencing (e.g. Internal Transcribed Spacer (ITS)), and whole genome shotgun sequencing. However, there are distinct advantages and disadvantages to each approach. Therefore, a combination of different methods described in the following paragraphs will help solve important current questions regarding what constitutes a core mycobiota and which fungi are transient or resident.
Many environmental, commensal, and pathogenic fungi can be cultured on standard media (11, 25). However, some fungi found in mammals require specific media conditions. Malassezia species, for example, fail to grow in the absence of fatty acids (17), and anaerobic fungi from ruminants require an anaerobic environment and specific additions like wheat straw for culture (27). A broad “culturomics” approach identified the highest number of fungi from human gut samples on Dixon medium, a complex medium that includes malt extract, ox bile and different fatty acids (11). In the gut, bacteria greatly outnumber fungi, which comprise about 0.1% of the gut microbiota (28). Isolation of fungi from gut samples therefore usually requires the addition of antibiotics (11, 25). Nevertheless, fungal species with a low abundance might not be recovered. colonies can be identified to the species level by species-specific PCR or amplification and sequencing of the ITS regions (11, 25). Species can also be identified via MALDI-TOF analysis, but identification is limited by the available databases, which are focused on pathogenic rather than commensal species (29, 30). One of the drawbacks of microbial culture is that it will identify all viable fungi, including fungal spores or transiently present fungi that might not be metabolically active in the gastrointestinal tract.

Visualization of Fungi

Culturing identifies viable organisms in a given sample. However, unless sampling is performed in specific sections (e.g. mucosa versus lumen), it gives no spatial information. Staining for fungi in fixed or frozen tissue samples can provide such spatial information. Fungi can be visualized in sectioned samples via immunostaining with fungal-specific antibodies (12, 31), soluble conjugated receptors (12), or fluorescence in situ hybridization (32, 33). These approaches are
limited by the specificity of antibody or probe used. However, they will identify fungal cells in a given sample and omit relic DNA.

**Metagenomic Analyses**

The specific technical and bioinformatic demands of mycobiome sequencing are expertly outlined elsewhere (34-36). Sequencing detects DNA of fungi present in a given sample regardless of whether they are culturable or not. Amplicon sequencing uses fungal-specific primers to amplify ITS or 18S regions of the rRNA gene locus, which contain hypervariable domains and allow for species discrimination (12, 37-39), analogous to 16S bacterial sequencing. This method illuminated a diverse mycobiome in humans and virtually all other species analyzed, for example mice (12, 32, 40-44), pigs (45), dogs (46), bees (47), and lizards (48). We will discuss the most frequently identified fungi of the human and mouse oral and gut mycobiome in the following chapters. Shortcomings of amplicon sequencing include amplification bias and the lack of comprehensive and fully annotated reference databases that take the complex fungal taxonomy into account (35, 39). Whole genome shotgun sequencing does not require amplification and can be used to analyze bacterial and fungal metagenomes simultaneously (28). An advantage is that publicly available datasets generated for bacteriome analysis can be re-analyzed for fungi (49). However, presence of fungi might be underestimated since fungal sequences are vastly outnumbered by bacterial sequences and some are not accurately identified as fungal due to the scarcity of available fungal genomes (36). A general drawback of DNA sequencing-based methods is the inability to discern between metabolically active organisms and relic DNA (36). The amount of relic DNA content in human feces seems currently unknown, but accounts for >40% of recovered sequences from soil samples (50). In the future, amplicon sequencing of the fungal ITS region derived from total RNA (36) or metatranscriptomic approaches might help to address this important question (51).
THE ORAL MYCOBIOTA

The gateway to the gastrointestinal tract is the oral cavity. Interactions of oral mycobiome members influence the local environment but can also have more distant effects. For example, *C. albicans* abundance in fecal matter is diminished when oral *C. albicans* abundance is reduced by more frequent brushing of teeth (52). Here, we will address which members of the oral mycobiota might constitute a core mycobiome, identify which are transient and which are colonizing, and highlight examples of interactions with fungi occurring in the oral cavity.

Members of the Oral Mycobiota

In their seminal study, Ghannoum and colleagues identified a total of 101 species in the oral cavity of 20 healthy individuals. However, 39 genera were present in only one subject and just 15 genera, including *Candida* and *Cladosporium*, were present in more than four individuals (53) (Fig. 1). Another study aimed to define the oral mycobiome found *Malassezia* to be the most prevalent genus in the oral mycobiome of six different healthy individuals (54). The oral mycobiome thus appears to be more subject-specific than the oral bacteriome, where 47% of bacterial OTUs were shared between three analyzed samples (55). Despite the high inter-individual variability, the core oral mycobiome within individuals seems largely stable, as it maintained a similar composition and relative abundance in human subjects over the course of a 30 week-long study (56). A recently published review article summarized the fungal species identified in different studies with both culturing and sequencing techniques (57). Interestingly, many of these fungi are known to be associated with plants, such as *Aureobasidium*, *Fusarium* and *Alternaria*; food commonly consumed, i.e. *Saccharomyces* and *Penicillium*; or found as...
mold both indoors and outdoors, such as *Aspergillus* species and *Cladosporium* (Fig. 1). This poses the question if all identified fungi are actively colonizing or if some are transiently present.

### Active and Transient Colonizers

Among the most commonly found fungi in the oral cavity are *Aspergillus* species, e.g. *niger*, and *Candida* species, such as *albicans*, *tropicalis*, and *parapsilosis* (58). A recent study identified them as the most abundant species in both healthy individuals and patients with periodontal disease (59). *Aspergillus* species are spore-forming filamentous fungi ubiquitously present in the environment (60). Even though *Aspergillus* species have been found as members of the oral mycobiome, pathological conditions due to *Aspergillus* are rare in the oral cavity and are usually restricted to the lungs and respiratory tract (61). On the other hand, *Candida* infections of the oral cavity are very common and affect up to 7% of infants, 30% of HIV patients and 20% of cancer patients (58). This discrepancy might be due to the possibility that *Aspergillus* is a transient member of the oral mycobiome, acquired via diet intake or inhalation. A report from 1966 showed that *Aspergillus flavus* was cultured from 60% of analyzed wheat flours and comprised 5.8% of the total fungal load (62). *Candida* species actively colonize the oral cavity and erupt in infections when conditions allow it. Both culture-dependent and culture-independent studies have identified *Candida* species as components of the oral mycobiome. *C. albicans* was present in 90% (63-65) or even 100% of analyzed subjects (66). Older age, poor oral hygiene and a fewer number of teeth are some of the associations found with increased level of colonization of *Candida* species (53). *C. albicans* has also been shown to take an active part in the biofilm formation and plaque virulence in combination with *Streptococcus mutans* (67) (Fig. 2). The oral mycobiome thus consists of both resident fungi and fungi that might only be transiently present. Mechanistic research on fungi in the oral cavity has been focused on the resident yeast *C. albicans*, which will be outlined in the next chapter. Other fungi of the oral
mycobiota, such as *Saccharomyces, Aspergillus, Penicillium* and *Malassezia*, might also represent active members of the oral microbiota, but their function has yet to be identified (53, 56).

**Examples of Fungal-Bacterial Interactions in the Oral Cavity**

**Protective Interactions**

Most of the studies investigating the cross-kingdom interactions of fungi and bacteria in the oral cavity have focused on models involving *C. albicans*. Usually existing in its commensal yeast form, *C. albicans* can be stimulated to form invasive hyphae (68). The oral commensal *Fusobacterium nucleatum* has been shown to adhere to both the yeast and hyphal forms of *C. albicans* (69, 70). This interaction limits *C. albicans* hyphal formation, thus reducing its ability to kill macrophages *in vitro* (71). The commensal *Aggregatibacter actinomycetemcomitans* inhibits biofilm production by *C. albicans* through the secretion of the quorum-sensing molecule autoinducer-2 (72) and limits polymicrobial biofilm formation by *C. albicans* and *Streptococcus mutans in vitro* (73) (Fig. 2). The oral bacterial microbiota thus has an active role in limiting the conversion of *C. albicans* yeast cells to invasive hyphae.

**Pathogenic Interactions**

Analysis of the salivary microbiome of older adults revealed that *Candida* species abundance is associated with decreased bacterial diversity and increased abundance of *Streptococcus* species (74). Various studies have investigated mutualistic interactions between *C. albicans* and species of *Streptococcus* that promote infection. Glucose-starved *C. albicans* has been shown to coaggregate with multiple *Streptococcus* species including *sanguis, gordonii, and oralis* (75) (Fig. 2). Further studies revealed that this coaggregation is mediated by cell wall polysaccharides, salivary proteins, and adhesins on the surface of *S. gordonii* (76-78). Adhesion
is also mediated by the receptors Als1p, Als3p, and Als5p on the surface of \textit{C. albicans} (79, 80).

An \textit{in vitro} study demonstrated that \textit{S. gordonii} can enhance \textit{C. albicans} biofilm formation (81, 82) (Fig. 2). \textit{S. mutans} also enhanced biofilm production in the oral cavity of infected mice through interactions between a glucosyltransferase secreted by \textit{S. mutans} and surface mannann expressed by \textit{C. albicans} (67, 83). Another study used an oral infection model of immunosuppressed mice to show that coinfection with \textit{S. oralis} enhances mucosal invasion of \textit{C. albicans} by synergistically promoting E-cadherin degradation (84). These studies demonstrate that some members of the oral bacterial microbiota, specifically the genus \textit{Streptococcus}, facilitate fungal overgrowth. More interactions between \textit{C. albicans} and \textit{Streptococcus} species have been reviewed in detail here (85).

\textbf{THE GUT MYCOBIOTA}

The vast majority of microorganisms in mammals can be found in the gut and fungi have emerged as an important component of the gut environment. Much research has therefore focused on understanding how the gut mycobiota is shaped and its interaction with human health.

\textbf{Development of the Gut Mycobiota}

The formation of the human mycobiota begins very early in life. Willis and colleagues recently suggested that fungal species might be present prior to birth and that \textit{C. albicans} specifically could be associated with pre-term delivery (86). Additional studies have shown that vaginal delivery allows vertical transmission of \textit{Candida} species from mother to infant (87, 88). Infants born by C-section harbor a bacterial microbiome similar to the mother’s skin microbiome (89). They could therefore also harbor higher \textit{Malassezia} species in their gastrointestinal tract, as this
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Fig. 1). After delivery, the gastrointestinal mycobiota is modulated by diet intake. For many infants, the main food source during the first months of age is breast milk. Boix-Amorós and colleagues found a core breast milk mycobiome, composed of Malassezia, Davidiella, Sistotrema and Penicillium, that was shared by study participants despite a varied geographical origin (93). Accordingly, the infant gut mycobiome is initially dominated by Malasseziales, most likely taken up through lactation. After the first 6 months of age, the infant gut mycobiome undergoes a dramatic change and is no longer dominated by Malasseziales but by Saccharomycetales instead (94). This change in mycobiome coincides with a change from breast milk to solid food. The gut microbiota further changes and matures during the development from childhood to adulthood (95). These changes are most likely driven by the development of the immune system and by the microorganisms that humans are exposed to through their diet and environment. Similar to oral mycobiome research, recent gut mycobiome research has focused on determining which fungal species are transient colonizers and which species are residents of the gastrointestinal tract.

Active and Transient Colonizers

Analysis of the Human Microbiome Project data determined Saccharomyces, Malassezia, Candida and Cyberlindnera as the four most abundant genera present in the human gut (Fig. 1). However, researchers found very high mycobiome variability between individuals and within individuals over time (96-98). Compared to the bacterial gut microbiome, the gut mycobiome thus seems to be less consistent and stable over time (96). The high fluctuation might in part be explained by fungi being introduced in the gastrointestinal lumen via diet intake and environment. Indeed, a standard human diet contains high levels of live fungi and fungal DNA (62, 99-102) and some fungal genera identified in the human GI tract are thought to lack the ability to grow at the temperature, pH, and low oxygen present in the gut environment (100).
Saccharomyces are ubiquitously present in the human diet, while Malassezia is the most abundant fungus colonizing the human skin (92, 103). Cyberlindnera is a food additive and most likely acquired through the diet (96), while Candida is the most identified fungus in the oral cavity (104). Indeed, a small-scale study showed that presence of Saccharomyces in human feces was directly dependent on diet intake while, as previously mentioned, C. albicans was associated with oral hygiene (52).

Current data thus suggests that some fungi in the human gastrointestinal tract can be classified as transient. However, evidence for true colonizers can also be found. C. albicans, Malassezia restricta, Cryptococcus neoformans and others have been shown to bloom in the gut under inflammatory conditions. This is particularly highlighted in patients affected with IBD where C. albicans increases in abundance during inflammation. However, it is not yet clear if C. albicans creates the inflammatory environment or if its increase in abundance is a consequence of inflammation (12, 105, 106). A unique phylum of resident fungi can be found in some herbivores but are not detected in humans or mice. Neocallimastigomycota are strictly anaerobic fungi that are present in all foregut fermenters (e.g. cows) and some hindgut fermenters (e.g. elephants), where they aid in the digestion of lignocellulose (107). Despite being an anaerobic environment, the human gut does not seem to support the colonization of strictly anaerobic fungi.

So, what proportion of the mycobiota is transiently present and what represents true residents? The bulk of research beginning to address this question has been performed in the mouse model, which we will focus on in the following section. Fungi in mice have been predominantly identified with culture-independent techniques (12, 32, 33, 40-44, 104). Interestingly, fungal sequences can not only be detected in feces, but also in mouse chow (12, 32, 41). Some of the identified genera, such as Aspergillus, Cladosporium, and Alternaria can be found in both feces and chow. However, other fungi, e.g. Candida, Fusarium, and Saccharomyces can be found only in feces and not in chow (12, 32), or their abundance is expanded in feces compared to chow (41). Similarly, a more recent study found that 80% of the fungal taxa identified in mice fed...
a standard diet were not present in the diet, and that 90% of fungal taxa identified in mice fed a high-fat diet were absent in the respective diet (40). These studies thus suggest that the composition of the mycobiota in the gut distinctly differs from the fungi present in the diet. Even though culture-independent techniques identified fungal species unique to feces, reports showing fungi cultured from mouse feces are rare and restricted to non-SPF conditions (43, 108). Possible reasons include (A) the relatively low abundance of fungi compared to bacteria or (B) the sampling site, as feces might harbor a different composition of live fungi compared to sites in the upper gastrointestinal tract (109). Interestingly, laboratory mice released in an outdoor environment show an increased alpha-diversity of the mycobiome and researchers were able to culture several fungal species from the mice’s feces. Most of the fungi that researchers were able to culture were *Aspergillus* species (43). This increase of fungi found in rewilded mice could be due to the presence of spores passing through the GI tract and/or an indication of live *Aspergilli* that have colonized the gut. The SPF environment of most laboratory mice might therefore be “too clean” to allow for acquisition of living fungi to colonize their gut. This is supported by the discovery that 22% of laboratory mice do not survive co-housing with pet store mice (110). Pet store mice harbor bacteria that are absent in laboratory mice and this might also be true for the fungal component of the microbiota. Collectively, studies in mice and humans support the idea that two mycobiomes are present in the mammalian gut: a transient mycobiome originating from diet intake and a resident mycobiome with persistently colonizing fungi. However, discriminating between transient fungi and active colonizers is still challenging. A combination of the different techniques outlined in our methods section, as well as other parameters, i.e. activation of the host immune response and interaction between bacteria and fungi, as postulated by Fiers and colleagues (102), will be essential to characterize the role of transient and resident fungi in the gut.

Bacterial-Fungal Interactions in the Gastrointestinal Tract
Recent studies have suggested that the mycobiota plays a role in maintaining homeostasis of the bacterial microbiota and influencing overall gut health. One study found that the administration of antifungal drugs to DSS-treated mice exasperates colitis and induces changes in the microbiome. Here, the microbiome undergoes an expansion of the bacterial genera *Hallella, Barnesiella, Bacteroides, Alistipes,* and *Lactobacillus* and a reduction of *Clostridium XIVa* and *Anaerostipes* (41). Another group found that the ingestion of the pathogenic fungus *Mucor circinelloides* by mice induced changes in the microbiota, notably with an increase in the genus *Bacteroides* and a decrease in *Akkermansia* (111). Another study demonstrated that *C. albicans* affects the re-colonization of the cecum by the microbiota in mice treated with antibiotics. The presence of the fungus increased the recovery of bacterial diversity, specifically the return of *Bacteroides* species. However, it also allowed colonization by the pathobiont *Enterococcus faecalis* and reduced colonization of probiotic *Lactobacillus* strains (112) (Fig. 2).

The mechanism of how *C. albicans* influences bacterial colonization is still unclear. A follow-up study revealed that antibiotic-treated, *C. albicans*-colonized mice showed reduced expression of specific immune genes but no visible changes in inflammation. These changes in expression could be limiting the host's ability to maintain microbial homeostasis, but there is still a possibility that *C. albicans* directly interacts with bacteria (113). A study that investigated differences in the microbiome between Japanese and Indian individuals proposed an interesting diet-fungal-bacterial interaction. The microbiome of the Indian participants showed a higher abundance of *Candida* and *Prevotella*. Since plants make up a major part of Indian diets, Pareek and colleagues went on to show that arabinoxylan, a plant polysaccharide, can be used as a growth factor by various *Candida* species. Finally, they showed that *Candida* supernatant enhances the growth of *Prevotella copri* and that prior colonization by *C. albicans* is required for the colonization of germ-free mice by *P. copri* (114). These studies indicate that interactions
between fungi and bacterial species influence gut homeostasis and are relevant to human health.

**Protective Interactions**

Specific cross-kingdom interactions between fungi and bacteria are currently being explored as a tool to maintain intestinal homeostasis. The yeast *Saccharomyces boulardii* has been extensively studied as a potential probiotic due to its protective effect against various bacterial gastrointestinal pathogens, including *Clostridium difficile*, *Helicobacter pylori*, *Vibrio cholerae*, *Salmonella enterica* serovar Typhimurium, *Shigella flexneri*, and *Escherichia coli* (115-122) (Fig. 2). Protection against *C. difficile* is at least partially due to the production of a protease by *S. boulardii* that degrades toxin A and B of *C. difficile* (123, 124). Protection against *V. cholerae* seems to involve the recognition of cholera toxin and subsequent activation of cyclic AMP signaling by *S. boulardii* (125). Even though *S. boulardii* has shown efficacy in a rat model of *V. cholerae* infection (119), this has yet to show clinical significance for humans (126). Both *E. coli* and *S. Typhimurium* bind to the surface of *S. boulardii*, potentially preventing adhesion to intestinal epithelial cells and thus allowing quicker excretion through fecal matter (127, 128). This interaction is inhibited by the addition of exogenous mannose, indicating that *E. coli* and *S. Typhimurium* are adhering to surface mannose residues present on *S. boulardii* (129). *S. boulardii* may also interact with commensal *Enterobacteriaceae* to alleviate DSS-induced colitis, as this protective effect is lost in mice treated with *Enterobacteriaceae*-depleting antibiotics (130). The depicted interactions underline the antipathogenic potential for commensal fungi, but the inverse also occurs, where commensal bacteria can protect against pathogenic fungi.

The most intensely studied examples of pathogenic fungi being antagonized by commensal bacteria involve *C. albicans*. Four probiotic strains, *Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus casei* GG, and *Bifidobacterium animalis*, have shown efficacy in limiting the severity of *C. albicans* infection in both immunocompromised and germ-free mice (131) (Fig.
Another probiotic mixture, consisting of *S. boulardii*, *L. acidophilus*, *Lactobacillus rhamnosus*, and *Bifidobacterium breve*, successfully inhibited the *in vitro* formation of polymicrobial biofilms containing *E. coli*, *Serratia marcescens*, and either *C. albicans* or *C. tropicalis* (132). These polymicrobial biofilms may be relevant to intestinal disease as *E. coli*, *S. marcescens*, and *C. albicans* have shown higher abundance in fecal samples from Crohn's disease patients (133). Various bacterial species have been shown to inhibit the transition of *C. albicans* to its invasive hyphal form. The widely studied probiotic *L. rhamnosus* GG produces an exopolysaccharide that limits hyphal formation and blocks *C. albicans* binding to intestinal epithelial cells *in vitro* (134). *L. rhamnosus* GG also inhibits *C. albicans* hyphae formation in liquid media via the peptidoglycan hydrolase Msp1, which degrades chitin present in the cell wall (135).

*Enterococcus faecalis* produces the bacteriocin EntV to inhibit *C. albicans* hyphae formation, reducing pathogenicity in a murine oropharyngeal candidiasis model infection (136). Studies have also reported that soluble factors produced by *E. coli* show antifungal activity against *C. albicans*. A soluble factor from the *E. coli* K-12 strain induced the death of *C. albicans* *in vitro* (137), and supernatant from an *E. coli* biofilm inhibited biofilm formation on polystyrene plates for a variety of *Candida* species (138). Furthermore, metabolites produced by a consortium of bacterial species derived from healthy human fecal samples effectively inhibited the growth of *C. albicans* in liquid culture. Species of *Roseburia* and *Bacteroides ovatus* were directly responsible for these antifungal effects (139). Interestingly, *C. albicans* also demonstrates probiotic properties by enhancing the growth of two strictly anaerobic commensal bacteria, *Bacteroides fragilis* and *Bacteroides vulgatus*, in liquid media. Possible mechanisms of this interaction are utilization of surface mannan as a carbon source or reduction of culture oxygen levels by *C. albicans* (140). Beneficial interactions between bacteria and fungi are continuously being explored as potential probiotic interventions for intestinal disease.

Pathogenic Interactions
Alternatively, interactions between fungal and bacterial commensals and pathogens have the potential to enhance pathogenesis. For example, mice treated with DSS to induce colitis showed increased disease when *C. albicans* is present. However, when mice were administered colistin to eliminate resident *Enterobacteriaceae*, the presence of *C. albicans* did not exasperate colitis severity. Supplementation with colistin-resistant *E. coli* restored *C. albicans* effect on DSS-induced colitis, suggesting that *Enterobacteriaceae* are required for *C. albicans*-mediated enhancement of colitis (130). Other studies have found that enterohemorrhagic *E. coli* enhances *C. albicans* invasion of intestinal epithelial cells *in vitro* (141). There is also evidence that *E. coli* strain O111:B4 enhances *C. albicans* infection of mice (142). *E. coli* 07KL was also found to enhance *C. albicans* attachment to epithelial cells *in vitro*, with a mechanism that likely involves bacterial pili (143). As mentioned in the previous section, the gut of Crohn’s disease patients can see an expansion of *E. coli*, *C. tropicalis*, and *S. marcescens*, which together have the ability to form polymicrobial biofilms *in vitro* (133). These studies thus underline that the interactions between *E. coli* and *Candida* species and their effect on pathogenesis are complex and strain dependent. *C. albicans* allows the growth of the strict anaerobe *C. difficile* under aerobic culture conditions (144). The ability of *C. albicans* to protect anaerobic bacteria under aerobic conditions is due to the rapid reduction of dissolved oxygen in the vicinity of the yeast (145). When examined in a mouse model of infection, *C. albicans* enhanced *C. difficile* pathogenicity when delivered orally one day prior to *C. difficile* infection (146). Another study found that the colonization of mice with *C. albicans* three weeks before *C. difficile* infection protected mice from infection (147). These two different experimental setups and outcomes indicate that the effect *C. albicans* has on *C. difficile* infection is dependent on the colonization state of *C. albicans*. Studies have also shown an interaction between *C. albicans* and *H. pylori* in gastric biopsy samples, where *H. pylori* was found within vacuoles in *C. albicans* cells (148, 149). It has been suggested that this behavior provides an environment that *H. pylori* can use to survive the low pH of the stomach (150) (Fig. 2). By analyzing whole stomachs of...
mice, Mason and colleagues show that antibiotic treatment allows for *C. albicans* colonization, triggering inflammation and inhibiting re-colonization by commensal *Lactobacillus* strains (151). Alternatively, the commensal yeast *S. cerevisiae* enhances the growth of the opportunistic pathogen *Acinetobacter baumannii* by producing ethanol. Furthermore, ethanol-stimulated *A. baumannii* shows enhanced pathogenicity in a *Caenorhabditis elegans* model of infection (152). It is important to keep these potentially detrimental interactions between pathogens, opportunistic pathogens, commensal bacteria, and fungi in mind when designing therapeutics involving probiotics.

**Antagonistic Interactions Between Pathogens**

There are also several antagonistic interactions between intestinal pathogens that do not have a clear benefit for intestinal health. One example is observed with *S. marcescens*, which employs a type VI secretion system to deliver antifungal toxins that kill both the yeast and hyphal form of *C. albicans* in liquid culture (153) (Fig. 2). *S. Typhimurium* also demonstrates a similar antifungal behavior by injecting type III secretion system effectors into *C. albicans*, blocking hyphal formation during *C. elegans* infection (154, 155). *A. baumannii* also demonstrates antifungal activity by binding to *C. albicans* filaments via OmpA and inducing apoptosis, preventing biofilm formation on polystyrene plates and limiting infection of *C. elegans* (156, 157). Conversely, *C. albicans* seems to express a mechanism to limit *A. baumannii* growth in *vitro* by producing the quorum-sensing molecule farnesol during late-stage biofilm formation (157). The previously mentioned symbiotic interaction provided by *C. albicans* to *C. difficile* is not reciprocated. The same study that found that *C. albicans* provides *C. difficile* with the means to grow under aerobic conditions also found that *C. difficile* inhibits *C. albicans* hyphal growth through the secretion of the small molecule *p*-cresol (144) (Fig. 2). These studies highlight bi-directional antagonistic interactions between pathogenic species of bacteria and fungi that are relevant to human health.
All bacterial-fungal interactions within the host occur in an environment that is ultimately regulated by the host immune response. The immunological changes stimulated by a specific microbial colonizer can have a profound effect on the intestinal environment, affecting a wide variety of microbial species already present. This is illustrated, for example, in a study that found that *Bacteroides thetaiotaomicron* stimulates expression of the innate immune genes hypoxia-inducible factor 1α (HIF-1α) and the antimicrobial peptide LL-37-CRAM and this differential expression provides colonization resistance against *C. albicans* in mice (158). Numerous studies have been performed focusing on the impact of both bacterial and fungal species on the host immune system and vice versa, as summarized previously (104, 159-165). We will focus on how the immune system recognizes fungi and some of the most recent studies on mycobiota and immune system interactions.

**Recognition of fungi by the immune system**

The prerequisite for the host to respond to fungi is the ability of cells, in particular immune cells, to identify and respond to different molecular patterns present on fungi. Among the pattern recognition receptors (PRRs) that can identify fungi are Toll-like receptors (TLRs), C-type lectin receptors (CLRs) and NOD-like receptors (NRLs). Fungal structures that are recognized by PRRs include surface polysaccharides, such as mannans or mannoproteins (TLR2, TLR4, Dectin-2, Mincle, DC-SIGN), β-glucans (TLR2, Dectin-1, NKp30), and unmethylated DNA (TLR9). Phagocytosed fungi can also activate NLRs, which leads to inflammasome formation and the production of the inflammatory protein IL-1β (109, 166-169). Mutations in the receptors highlight the importance of a proper recognition of fungi by the immune system. Mutations in the
gene encoding for Dectin-1 have been associated with increased *C. tropicalis* invasion in mice and exacerbated colitis in both mice and humans (12). Mutations in Dectin-2 were found to be associated with increased *C. glabrata* infections due to a deficient immune response to the fungus (170). Lack of TLR4 and TLR2 responses were shown to affect disseminated candidiasis in mice: lack of TLR4 caused an increased *C. albicans* kidney burden, while blocking of TLR2 inhibited the production of inflammatory cytokines such as TNF-α and IL-1β (171). Several other receptors are involved in the recognition of fungi. These include the recently identified MelLec, a CLR able to bind melanin on *A. fumigatus* conidia (172), soluble receptors such as pentraxins and mannose-binding lectin (MBL), involved in the recognition of galactomannan and mannan respectively, as well as the intracellular RIG-I-like receptor (RLR) MDA5 which was found to be involved in the immune response to systemic *C. albicans* infection (167, 173, 174).

**Immune system-mycobiota interaction**

Much emphasis has been placed on understanding the roles of the mycobiota in shaping the immune system. A recent study highlighted the important contribution of fungi in the maturation of the immune system. The authors showed that fungi colonizing the gut of mice kept in a natural outdoor environment were sufficient to induce an increase in circulating granulocytes to a level more similar to humans than laboratory mice (43). This finding expanded on results showing that mice colonized with a “wild mouse microbiota” would respond to immunotherapy in a manner more similar to humans (175). Fungi are not only important during homeostatic conditions but are also necessary for the development of a healthy immune system. Most of the research performed tries to understand the involvement of the mycobiota in the development and origin of inflammatory and pathogenic conditions. *Candida* and *Malassezia* are among the fungal genera that have been most studied in this context. *Candida* species, particularly *C.*
*C. albicans*, are known to be able to exacerbate gut inflammation (161). Fungal dysbiosis and increased *C. albicans* colonization were identified in association with IBD in human patients (176). In a mouse model of DSS-induced colitis, the presence of *C. albicans* also worsened local and systemic inflammation (177). Like *C. albicans*, *M. restricta* was shown to increase disease severity in DSS-treated mice. Increased relative abundance of *M. restricta* in the colon of Crohn’s Disease patients was linked to a mutation in the CARD9 gene, CARD$^{S12N}$, previously associated with the onset of IBD (105). This ability of *Malassezia* to elicit inflammation was later connected to the activation of the NLRP3 inflammasome (178). Dysbiosis of the gut mycobiota can also affect distal organs. A recent study found that gut dysbiosis, specifically increased abundance of *Malassezia* species, promoted pancreatic ductal adenocarcinoma development through the activation of the complement cascade via the engagement of the mannose-binding lectin (33). Gut dysbiosis has also been linked to lung inflammation. During gut inflammation, *C. albicans* was shown to induce the generation of Th17 cells cross-reactive against the airborne pathogen *A. fumigatus*, thus contributing to the exacerbation of allergic bronchopulmonary aspergillosis (179). Similarly, CX3CR1$^+$ mononuclear phagocytes, present in the lamina propria and involved in trafficking bacteria from the gut to mesenteric lymph nodes (180), were identified to play an important role in the immunity against fungi in the gut (181) and are able to create a gut-lung axis that exacerbates allergic airway disease following gut fungal dysbiosis (182). Another example of interconnection between the gut and lungs is the expansion of *Wallemia mellicola* in the gut following antibiotic treatment. This intestinal expansion exacerbates lung inflammation by increasing eosinophil recruitment in a mouse model of allergic airway disease (44). A recently published study finally highlighted the importance of a balanced immune response to control fungal infections. Th17 immune responses are known to be important during mucocutaneous fungal infections, especially *C. albicans* infections (162). However, Break and
colleagues showed that in mice and human patients with mutation in the gene Aire, that had an intact Th17 response, an excessive IFN-γ production by T cells at the mucosal level was the cause of increased susceptibility to chronic mucocutaneous candidiasis (183). Future studies will continue to expand our knowledge on the role of fungi during homeostatic and pathological conditions and dissect their role in the development of a balanced immune system.

CONCLUSION AND OUTLOOK

Mycobiome research is a rapidly expanding scientific field, but many questions are currently still unanswered. Due to the high inter- and intra-individual variability it is unclear if core mycobiomes can be defined. Future research will expand our knowledge on which fungi are resident and which are transiently present in the gastrointestinal tract. However, it is indisputable that the mycobiome fulfills crucial roles. Irrespective of their ability to colonize, fungi interact with and train the immune system and contribute to gastrointestinal homeostasis. Analogous to bacteriome research, mycobiome research is also moving from describing composition to ascribing function. Highly interesting mechanisms of how specific gut commensal fungi modulate immune responses and interact with bacteria are beginning to emerge. Future research directions include the characterization of the fungal metabolome in the gastrointestinal tract to identify which products are produced by fungi and how are they influence the microbiome and the host. A recent analysis of the metabolome of differently colonized gnotobiotic mice found that fungi significantly contributed to microbial ecology and host immune functionality but contributed only a small extent to the overall gut metabolome (175). More research with an extended spectrum of commensal gut fungi and additional models will be needed to define the fungal metabolome and the role of fungi in the gut ecosystem.
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REFERENCES

1. Hamm PS, Taylor JW, Cook JA, Natvig DO. 2020. Decades-old studies of fungi associated with mammalian lungs and modern DNA sequencing approaches help define the nature of the lung mycobiome. PLoS Pathog 16:e1008684.

2. Kramer R, Sauer-Heilborn A, Welte T, Guzman CA, Abraham WR, Hofle MG. 2015. Cohort Study of Airway Mycobiome in Adult Cystic Fibrosis Patients: Differences in Community Structure between Fungi and Bacteria Reveal Predominance of Transient Fungal Elements. J Clin Microbiol 53:2900-7.

3. Rick EM, Woolnough KF, Seear PJ, Fairs A, Satchwell J, Richardson M, Monteiro WR, Craner M, Bourne M, Wardlaw AJ, Pashley CH. 2020. The airway fungal microbiome in asthma. Clin Exp Allergy doi:10.1111/cea.13722.

4. Bradford LL, Ravel J. 2017. The vaginal myco biome: A contemporary perspective on fungi in women’s health and diseases. Virulence 8:342-351.
563  5. Ward TL, Dominguez-Bello MG, Heisel T, Al-Ghalith G, Knights D, Gale CA. 2018. Development of the Human Mycobiome over the First Month of Life and across Body Sites. mSystems 3.

564  6. Ackerman AL, Underhill DM. 2017. The mycobiome of the human urinary tract: potential roles for fungi in urology. Ann Transl Med 5:31.

565  7. Ackerman AL, Chai TC. 2019. The Bladder is Not Sterile: an Update on the Urinary Microbiome. Curr Bladder Dysfunct Rep 14:331-341.

566  8. Baumgardner DJ. 2019. Oral Fungal Microbiota: To Thrush and Beyond. J Patient Cent Res Rev 6:252-261.

567  9. Wu L, Zeng T, Deligios M, Milanesi L, Langille MGI, Zinellu A, Rubino S, Carru C, Kelvin DJ. 2020. Age-Related Variation of Bacterial and Fungal Communities in Different Body Habitats across the Young, Elderly, and Centenarians in Sardinia. mSphere 5.

568  10. Bandara H, Panduwawala CP, Samaranayake LP. 2019. Biodiversity of the human oral mycobiome in health and disease. Oral Dis 25:363-371.

569  11. Hamad I, Ranque S, Azhar EI, Yasir M, Jiman-Fatani AA, Tissot-Dupont H, Raoult D, Bittar F. 2017. Culturomics and Amplicon-based Metagenomic Approaches for the Study of Fungal Population in Human Gut Microbiota. Sci Rep 7:16788.

570  12. Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, Strom SP, Brown J, Becker CA, Fleshner PR, Dubinsky M, Rotter JL, Wang HL, McGovern DP, Brown GD, Underhill DM. 2012. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science 336:1314-7.

571  13. Keum HL, Kim H, Kim HJ, Park T, Kim S, An S, Sul WJ. 2020. Structures of the Skin Microbiome and Mycobiome Depending on Skin Sensitivity. Microorganisms 8.
14. Zhu T, Duan YY, Kong FQ, Galzote C, Quan ZX. 2020. Dynamics of Skin Mycobiome in Infants. Front Microbiol 11:1790.

15. Leong C, Schmid B, Toi MJ, Wang J, Irudayaswamy AS, Goh JPZ, Bosshard PP, Glatz M, Dawson TL, Jr. 2019. Geographical and Ethnic Differences Influence Culturable Commensal Yeast Diversity on Healthy Skin. Front Microbiol 10:1891.

16. Heisel T, Nyaribo L, Sadowsky MJ, Gale CA. 2019. Breastmilk and NICU surfaces are potential sources of fungi for infant mycobiomes. Fungal Genet Biol 128:29-35.

17. Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegraki A. 2012. The Malassezia genus in skin and systemic diseases. Clin Microbiol Rev 25:106-41.

18. Heinekamp T, Schmidt H, Lapp K, Pahtz V, Shopova I, Koster-Eiserfunke N, Kruger T, Kniemeyer O, Brakhage AA. 2015. Interference of Aspergillus fumigatus with the immune response. Semin Immunopathol 37:141-52.

19. Baltussen TJH, Zoll J, Verweij PE, Melchers WJG. 2020. Molecular Mechanisms of Conidial Germination in Aspergillus spp. Microbiol Mol Biol Rev 84.

20. Mba IE, Nweze EI. 2020. Mechanism of Candida pathogenesis: revisiting the vital drivers. Eur J Clin Microbiol Infect Dis 39:1797-1819.

21. Köhler JR, Hube B, Puccia R, Casadevall A, Perfect JR. 2017. Fungi that Infect Humans. Microbiol Spectr 5.

22. d’Enfert C, Kaune AK, Alaban LR, Chakraborthy S, Cole N, Delavy M, Kosmala D, Marsaux B, Frois-Martins R, Morelli M, Rosati D, Valentine M, Xie Z, Emritoll Y, Warn PA, Bequet F, Bougnoux ME, Bornes S, Gresnigt MS, Hube B, Jacobsen ID, Legrand M, Leibundgut-Landmann S, Manichanh C, Munro CA, Netea MG, Queiroz K, Roget K, Thomas V, Thoral C, Van den Abbeele P, Walker AW, Brown AJP. 2020. The impact of
the Fungus-Host-Microbiota interplay upon *Candida albicans* infections: current knowledge and new perspectives. FEMS Microbiol Rev doi:10.1093/femsre/fuaa060.

23. Jabra-Rizk MA, Kong EF, Tsui C, Nguyen MH, Clancy CJ, Fidel PL, Jr., Noverr M. 2016. *Candida albicans* Pathogenesis: Fitting within the Host-Microbe Damage Response Framework. Infect Immun 84:2724-39.

24. Chen Y, Chen Z, Guo R, Chen N, Lu H, Huang S, Wang J, Li L. 2011. Correlation between gastrointestinal fungi and varying degrees of chronic hepatitis B virus infection. Diagn Microbiol Infect Dis 70:492-8.

25. Scanlan PD, Marchesi JR. 2008. Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. ISME J 2:1183-93.

26. Prohic A, Jovovic Sadikovic T, Krupalja-Fazlic M, Kuskunovic-Vlahovljak S. 2016. *Malassezia* species in healthy skin and in dermatological conditions. Int J Dermatol 55:494-504.

27. Griffith GW, Ozkose MK, Theodorou MK, Davies DR. 2009. Diversity of anaerobic fungal populations in cattle revealed by selective enrichment culture using different carbon sources. Fungal Ecology 2:87-97.

28. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, et al. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59-65.
29. Lu JJ, Lo HJ, Lee CH, Chen MJ, Lin CC, Chen YZ, Tsai MH, Wang SH. 2021. The Use of MALDI-TOF Mass Spectrometry to Analyze Commensal Oral Yeasts in Nursing Home Residents. Microorganisms 9.

30. Patel R. 2019. A Moldy Application of MALDI: MALDI-ToF Mass Spectrometry for Fungal Identification. J Fungi (Basel) 5.

31. Bohm L, Torsin S, Tint SH, Eckstein MT, Ludwig T, Perez JC. 2017. The yeast form of the fungus *Candida albicans* promotes persistence in the gut of gnotobiotic mice. PLoS Pathog 13:e1006699.

32. Scupham AJ, Presley LL, Wei B, Bent E, Griffith N, McPherson M, Zhu F, Oluwadara O, Rao N, Braun J, Borneman J. 2006. Abundant and diverse fungal microbiota in the murine intestine. Appl Environ Microbiol 72:793-801.

33. Aykut B, Pushalkar S, Chen R, Li Q, Abengoza R, Kim JI, Shadaloey SA, Wu D, Preiss P, Verma N, Guo Y, Saxena A, Bardhan M, Diskin B, Wang W, Leinwand J, Kurz E, Kochen Rossi JA, Hundeyin M, Zambrinis C, Li X, Saxena D, Miller G. 2019. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. Nature 574:264-267.

34. Halwachs B, Madhusudhan N, Krause R, Nilsson RH, Moissl-Eichinger C, Hogenauer C, Thallinger GG, Gorkiewicz G. 2017. Critical Issues in Mycobiota Analysis. Front Microbiol 8:180.

35. Tang J, Iliev ID, Brown J, Underhill DM, Funari VA. 2015. Mycobiome: Approaches to analysis of intestinal fungi. J Immunol Methods 421:112-121.

36. Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 17:95-109.
37. Yang RH, Su JH, Shang JJ, Wu YY, Li Y, Bao DP, Yao YJ. 2018. Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. PLoS One 13:e0206428.

38. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding C, Fungal Barcoding Consortium Author L. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A 109:6241-6.

39. Frau A, Kenny JG, Lenzi L, Campbell BJ, Ijaz UZ, Duckworth CA, Burkitt MD, Hall N, Anson J, Darby AC, Probert CSJ. 2019. DNA extraction and amplicon production strategies deeply influence the outcome of gut mycobiome studies. Sci Rep 9:9328.

40. Heisel T, Montassier E, Johnson A, Al-Ghalith G, Lin YW, Wei LN, Knights D, Gale CA. 2017. High-Fat Diet Changes Fungal Microbiomes and Interkingdom Relationships in the Murine Gut. mSphere 2.

41. Qiu X, Zhang F, Yang X, Wu N, Jiang W, Li X, Li X, Liu Y. 2015. Changes in the composition of intestinal fungi and their role in mice with dextran sulfate sodium-induced colitis. Scientific Reports 5:10416.

42. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, McCulloch JA, Anastasakis DG, Sarshad AA, Leonard I, Collins N, Blatter JA, Han SJ, Tamoutounour S, Potapova S, Foster St Claire MB, Yuan W, Sen SK, Dreier MS, Hild B, Hafner M, Wang D, Iliev ID, Belkaid Y, Trinchieri G, Rehmann B. 2019. Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science 365.

43. Yeung F, Chen YH, Lin JD, Leung JM, McCauley C, Devlin JC, Hansen C, Cronkite A, Stephens Z, Drake-Dunn C, Fulmer Y, Shopsin B, Ruggles KV, Round JL, Loke P,
Graham AL, Cadwell K. 2020. Altered Immunity of Laboratory Mice in the Natural Environment Is Associated with Fungal Colonization. Cell Host Microbe 27:809-822.e6.

Skalski JH, Limon JJ, Sharma P, Gargus MD, Nguyen C, Tang J, Coelho AL, Hogaboam CM, Crother TR, Underhill DM. 2018. Expansion of commensal fungus Wallemia mellicola in the gastrointestinal mycobiota enhances the severity of allergic airway disease in mice. PLoS Pathog 14:e1007260.

Arfken AM, Frey JF, Summers KL. 2020. Temporal Dynamics of the Gut Bacteriome and Mycobiome in the Weanling Pig. Microorganisms 8.

Foster ML, Dowd SE, Stephenson C, Steiner JM, Suchodolski JS. 2013. Characterization of the fungal microbiome (mycobiome) in fecal samples from dogs. Vet Med Int 2013:658373.

Yun JH, Jung MJ, Kim PS, Bae JW. 2018. Social status shapes the bacterial and fungal gut communities of the honey bee. Sci Rep 8:2019.

Montoya-Ciriaco N, Gomez-Acata S, Munoz-Arenas LC, Dendooven L, Estrada-Torres A, Diaz de la Vega-Perez AH, Navarro-Noya YE. 2020. Dietary effects on gut microbiota of the mesquite lizard Sceloporus grammicus (Wiegmann, 1828) across different altitudes. Microbiome 8:6.

Donovan PD, Gonzalez G, Higgins DG, Butler G, Ito K. 2018. Identification of fungi in shotgun metagenomics datasets. PLoS One 13:e0192898.

Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N. 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat Microbiol 2:16242.
51. Marcelino VR, Irinyi L, Eden JS, Meyer W, Holmes EC, Sorrell TC. 2019. Metatranscriptomics as a tool to identify fungal species and subspecies in mixed communities - a proof of concept under laboratory conditions. IMA Fungus 10:12.

52. Auchtung TA, Fofanova TY, Stewart CJ, Nash AK, Wong MC, Gesell JR, Auchtung JM, Ajami NJ, Petrosino JF. 2018. Investigating Colonization of the Healthy Adult Gastrointestinal Tract by Fungi. mSphere 3.

53. Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, Gillevet PM. 2010. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 6:e1000713.

54. Dupuy AK, David MS, Li L, Heider TN, Peterson JD, Montano EA, Dongari-Bagtzoglou A, Diaz PI, Strausbaugh LD. 2014. Redefining the human oral mycobiome with improved practices in amplicon-based taxonomy: discovery of Malassezia as a prominent commensal. PLoS One 9:e90899.

55. Zaura E, Keijser BJ, Huse SM, Crielaard W. 2009. Defining the healthy "core microbiome" of oral microbial communities. BMC Microbiol 9:259.

56. Monteiro-da-Silva F, Araujo R, Sampaio-Maia B. 2014. Interindividual variability and intraindividual stability of oral fungal microbiota over time. Med Mycol 52:498-505.

57. Diaz PI, Hong BY, Dupuy AK, Strausbaugh LD. 2017. Mining the oral mycobiome: Methods, components, and meaning. Virulence 8:313-323.

58. Patil S, Rao RS, Majumdar B, Anil S. 2015. Clinical Appearance of Oral Candida Infection and Therapeutic Strategies. Front Microbiol 6:1391.

59. Peters BA, Wu J, Hayes RB, Ahn J. 2017. The oral fungal mycobiome: characteristics and relation to periodontitis in a pilot study. BMC Microbiol 17:157.
Mousavi B, Hedayati MT, Hedayati N, Ilkit M, Syedmousavi S. 2016. *Aspergillus* species in indoor environments and their possible occupational and public health hazards. Curr Med Mycol 2:36-42.

Cho H, Lee KH, Colquhoun AN, Evans SA. 2010. Invasive oral aspergillosis in a patient with acute myeloid leukaemia. Aust Dent J 55:214-8.

Graves RR, Hesselteine CW. 1966. Fungi in flour and refrigerated dough products. Mycopathol Mycol Appl 29:277-90.

O’Connell LM, Santos R, Springer G, Burne RA, Nascimento MM, Richards VP. 2020. Site-Specific Profiling of the Dental Mycobiome Reveals Strong Taxonomic Shifts during Progression of Early-Childhood Caries. Appl Environ Microbiol 86.

Li Y, Wang K, Zhang B, Tu Q, Yao Y, Cui B, Ren B, He J, Shen X, Van Nostrand JD, Zhou J, Shi W, Xiao L, Lu C, Zhou X. 2019. Salivary mycobiome dysbiosis and its potential impact on bacteriome shifts and host immunity in oral lichen planus. Int J Oral Sci 11:13.

Persoon IF, Buijs MJ, Özok AR, Crielaard W, Krom BP, Zaura E, Brandt BW. 2017. The mycobiome of root canal infections is correlated to the bacteriome. Clin Oral Investig 21:1871-1881.

Fechney JM, Browne GV, Prabhu N, Irinyi L, Meyer W, Hughes T, Bockmann M, Townsend G, Salehi H, Adler CJ. 2019. Preliminary study of the oral mycobiome of children with and without dental caries. J Oral Microbiol 11:1536182.

Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai C-H, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, Koo H. 2014. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. Infection and immunity 82:1968-1981.
68. Gow NAR, van de Veerdonk FL, Brown AJP, Netea MG. 2012. *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. Nature Reviews Microbiology 10:112-122.

69. Grimaudo NJ, Nesbitt WE. 1997. Coaggregation of *Candida albicans* with oral *Fusobacterium* species. Oral Microbiology and Immunology 12:168-173.

70. Wu T, Cen L, Kaplan C, Zhou X, Lux R, Shi W, He X. 2015. Cellular Components Mediating Coadherence of *Candida albicans* and *Fusobacterium nucleatum*. Journal of dental research 94:1432-1438.

71. Bor B, Cen L, Agnello M, Shi W, He X. 2016. Morphological and physiological changes induced by contact-dependent interaction between *Candida albicans* and *Fusobacterium nucleatum*. Scientific reports 6:27956-27956.

72. Bachtiar EW, Bachtiar BM, Jarosz LM, Amir LR, Sunarto H, Ganin H, Meijler MM, Krom BP. 2014. AI-2 of *Aggregatibacter actinomycetemcomitans* inhibits *Candida albicans* biofilm formation. Frontiers in cellular and infection microbiology 4:94-94.

73. Bachtiar EW, Bachtiar BM. 2020. Effect of cell-free spent media prepared from *Aggregatibacter actinomycetemcomitans* on the growth of *Candida albicans* and *Streptococcus mutans* in co-species biofilms. European Journal of Oral Sciences 128:395-404.

74. Kraneveld EA, Buijs MJ, Bonder MJ, Visser M, Keijser BJF, Crielaard W, Zaura E. 2012. The relation between oral *Candida* load and bacterial microbiome profiles in Dutch older adults. PloS one 7:e42770-e42770.

75. Jenkinson HF, Lala HC, Shepherd MG. 1990. Coaggregation of *Streptococcus sanguis* and other streptococci with *Candida albicans*. Infection and immunity 58:1429-1436.
76. Holmes AR, Gopal PK, Jenkinson HF. 1995. Adherence of *Candida albicans* to a cell surface polysaccharide receptor on *Streptococcus gordonii*. Infection and immunity 63:1827-1834.

77. O’Sullivan JM, Jenkinson HF, Cannon RD. 2000. Adhesion of *Candida albicans* to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface. Microbiology 146:41-48.

78. Holmes AR, McNab R, Jenkinson HF. 1996. *Candida albicans* binding to the oral bacterium *Streptococcus gordonii* involves multiple adhesin-receptor interactions. Infection and immunity 64:4680-4685.

79. Klotz SA, Gaur NK, De Armond R, Sheppard D, Khardori N, Edwards JE, Jr., Lipke PN, El-Azizi M. 2007. *Candida albicans* Als proteins mediate aggregation with bacteria and yeasts. Medical Mycology 45:363-370.

80. Silverman RJ, Nobbs AH, Vickerman MM, Barbour ME, Jenkinson HF. 2010. Interaction of *Candida albicans* Cell Wall Als3 Protein with *Streptococcus gordonii* SspB Adhesin Promotes Development of Mixed-Species Communities. Infection and Immunity 78:4644-4652.

81. Bamford CV, d’Mello A, Nobbs AH, Dutton LC, Vickerman MM, Jenkinson HF. 2009. *Streptococcus gordonii* Modulates *Candida albicans* Biofilm Formation through Intergeneric Communication. Infection and Immunity 77:3696-3704.

82. Montelongo-Jauregui D, Saville SP, Lopez-Ribot JL. 2019. Contributions of *Candida albicans* Dimorphism, Adhesive Interactions, and Extracellular Matrix to the Formation of Dual-Species Biofilms with *Streptococcus gordonii*. mBio 10:e01179-19.
83. Hwang G, Liu Y, Kim D, Li Y, Krysan DJ, Koo H. 2017. *Candida albicans* mannans mediate *Streptococcus mutans* exoenzyme GtfB binding to modulate cross-kingdom biofilm development *in vivo*. PLOS Pathogens 13:e1006407.

84. Xu H, Sobue T, Bertolini M, Thompson A, Dongari-Bagtzoglou A. 2016. *Streptococcus oralis* and *Candida albicans* Synergistically Activate μ-Calpain to Degrade E-cadherin From Oral Epithelial Junctions. The Journal of infectious diseases 214:925-934.

85. Förster TM, Mogavero S, Dräger A, Graf K, Polke M, Jacobsen ID, Hube B. 2016. Enemies and brothers in arms: *Candida albicans* and gram-positive bacteria. Cellular Microbiology 18:1709-1715.

86. Willis KA, Purvis JH, Myers ED, Aziz MM, Karabayir I, Gomes CK, Peters BM, Akbilgic O, Talati AJ, Pierre JF. 2019. Fungi form interkingdom microbial communities in the primordial human gut that develop with gestational age. Faseb J 33:12825-12837.

87. Al-Rusan RM, Darwazeh AM, Latafeh IM. 2017. The relationship of *Candida* colonization of the oral and vaginal mucosae of mothers and oral mucosae of their newborns at birth. Oral Surg Oral Med Oral Pathol Oral Radiol 123:459-463.

88. Waggoner-Fountain LA, Walker MW, Hollis RJ, Pfaller MA, Ferguson JE, 2nd, Wenzel RP, Donowitz LG. 1996. Vertical and horizontal transmission of unique *Candida* species to premature newborns. Clin Infect Dis 22:803-8.

89. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 107:11971-5.

90. Nagata R, Nagano H, Ogishima D, Nakamura Y, Hiruma M, Sugita T. 2012. Transmission of the major skin microbiota, *Malassezia*, from mother to neonate. Pediatr Int 54:350-5.
91. Ward TL, Knights D, Gale CA. 2017. Infant fungal communities: current knowledge and research opportunities. BMC Med 15:30.

92. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, Schoenfeld D, Nomicos E, Park M, Kong HH, Segre JA. 2013. Topographic diversity of fungal and bacterial communities in human skin. Nature 498:367-70.

93. Boix-Amorós A, Puente-Sánchez F, du Toit E, Linderborg KM, Zhang Y, Yang B, Salminen S, Isolauri E, Tamames J, Mira A, Collado MC. 2019. Mycobiome Profiles in Breast Milk from Healthy Women Depend on Mode of Delivery, Geographic Location, and Interaction with Bacteria. Appl Environ Microbiol 85.

94. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosh D, Panzer AR, LaMere B, Rackaityte E, Lukacs NW, Wegienka G, Boushey HA, Ownby DR, Zoratti EM, Levin AM, Johnson CC, Lynch SV. 2016. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. Nat Med 22:1187-1191.

95. Nagpal R, Mainali R, Ahmadi S, Wang S, Singh R, Kavanagh K, Kitzman DW, Kushugulova A, Marotta F, Yadav H. 2018. Gut microbiome and aging: Physiological and mechanistic insights. Nutr Healthy Aging 4:267-285.

96. Nash AK, Auchtung TA, Wong MC, Smith DP, Gesell JR, Ross MC, Stewart CJ, Metcalf GA, Muzny DM, Gibbs RA, Ajami NJ, Petrosino JF. 2017. The gut mycobiome of the Human Microbiome Project healthy cohort. Microbiome 5:153.

97. Consortium THMP. 2012. A framework for human microbiome research. Nature 486:215-21.

98. Consortium THMP. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486:207-14.
99. Tournas VH, Niazi NS. 2017. Potentially toxigenic fungi from selected grains and grain products. Journal of Food Safety 38.

100. Suhr MJ, Hallen-Adams HE. 2015. The human gut mycobiome: pitfalls and potentials—a mycologist’s perspective. Mycologia 107:1057-73.

101. Raimondi S, Amaretti A, Gozzoli C, Simone M, Righini L, Candelieri F, Brun P, Ardizzoni A, Colombari B, Paulone S, Castagliuolo I, Cavalieri D, Blasi E, Rossi M, Peppoloni S. 2019. Longitudinal Survey of Fungi in the Human Gut: ITS Profiling, Phenotyping, and Colonization. Front Microbiol 10:1575.

102. Fiers WD, Gao IH, Iliev ID. 2019. Gut mycobiota under scrutiny: fungal symbionts or environmental transients? Curr Opin Microbiol 50:79-86.

103. Oh J, Freeman AF, Park M, Sokolic R, Candotti F, Holland SM, Segre JA, Kong HH. 2013. The altered landscape of the human skin microbiome in patients with primary immunodeficiencies. Genome Res 23:2103-14.

104. Underhill DM, Iliev ID. 2014. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol 14:405-16.

105. Limon JJ, Tang J, Li D, Wolf AJ, Michelsen KS, Funari V, Gargus M, Nguyen C, Sharma P, Maymi VI, Iliev ID, Skalski JH, Brown J, Landers C, Borneman J, Braun J, Targan SR, McGovern DPB, Underhill DM. 2019. Malassezia Is Associated with Crohn’s Disease and Exacerbates Colitis in Mouse Models. Cell Host Microbe 25:377-388.e6.

106. Li Q, Wang C, Tang C, He Q, Li N, Li J. 2014. Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in Crohn’s disease. J Clin Gastroenterol 48:513-23.

107. Gruninger RJ, Puniya AK, Callaghan TM, Edwards JE, Youssef N, Dagar SS, Fliegerova K, Griffith GW, Forster R, Tsang A, McAllister T, Elshahed MS. 2014. Anaerobic fungi
(phylum Neocallimastigomycota): advances in understanding their taxonomy, life cycle, ecology, role and biotechnological potential. FEMS Microbiol Ecol 90:1-17.

108. Stejskal V, Hubert J, Kubitova A, Vanova M. 2005. Fungi associated with rodent feces in stored grain environment in the Czech Republic. Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection 112:98-102.

109. Richard ML, Sokol H. 2019. The gut mycobiota: insights into analysis, environmental interactions and role in gastrointestinal diseases. Nat Rev Gastroenterol Hepatol 16:331-345.

110. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA, Thompson EA, Fraser KA, Rosato PC, Filali-Mouhim A, Sekaly RP, Jenkins MK, Vezys V, Haining WN, Jameson SC, Masopust D. 2016. Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature 532:512-6.

111. Mueller KD, Zhang H, Serrano CR, Billmyre RB, Huh EY, Wiemann P, Keller NP, Wang Y, Heitman J, Lee SC. 2019. Gastrointestinal microbiota alteration induced by Mucor circinelloides in a murine model. Journal of microbiology (Seoul, Korea) 57:509-520.

112. Mason KL, Erb Downward JR, Mason KD, Falkowski NR, Eaton KA, Kao JY, Young VB, Huffnagle GB. 2012. Candida albicans and bacterial microbiota interactions in the cecum during recolonization following broad-spectrum antibiotic therapy. Infect Immun 80:3371-80.

113. Erb Downward JR, Falkowski NR, Mason KL, Muraglia R, Huffnagle GB. 2013. Modulation of post-antibiotic bacterial community reassembly and host response by Candida albicans. Scientific reports 3:2191-2191.

114. Pareek S, Kurakawa T, Das B, Motooka D, Nakaya S, Rongsen-Chandola T, Goyal N, Kayama H, Dodd D, Okumura R, Maeda Y, Fujimoto K, Nii T, Ogawa T, Iida T, Bhandari...
N, Kida T, Nakamura S, Nair GB, Takeda K. 2019. Comparison of Japanese and Indian intestinal microbiota shows diet-dependent interaction between bacteria and fungi. npj Biofilms and Microbiomes 5:37.

Kelesidis T, Pothoulakis C. 2012. Efficacy and safety of the probiotic Saccharomyces boulardii for the prevention and therapy of gastrointestinal disorders. Therapeutic advances in gastroenterology 5:111-125.

Surawicz CM, McFarland LV, Greenberg RN, Rubin M, Fekety R, Mulligan ME, Garcia RJ, Brandmarker S, Bowen K, Borjal D, Elmer GW. 2000. The Search for a Better Treatment for Recurrent Clostridium difficile Disease: Use of High-Dose Vancomycin Combined with Saccharomyces boulardii. Clinical Infectious Diseases 31:1012-1017.

Szajewska H, Horvath A, Piwowarczyk A. 2010. Meta-analysis: the effects of Saccharomyces boulardii supplementation on Helicobacter pylori eradication rates and side effects during treatment. Aliment Pharmacol Ther 32:1069-79.

Zhou BG, Chen LX, Li B, Wan LY, Ai YW. 2019. Saccharomyces boulardii as an adjuvant therapy for Helicobacter pylori eradication: A systematic review and meta-analysis with trial sequential analysis. Helicobacter 24:e12651.

Czerucka D, Rampal P. 2002. Experimental effects of Saccharomyces boulardii on diarrheal pathogens. Microbes and Infection 4:733-739.

Martins FS, Dalmasso G, Arantes RME, Doye A, Lemichez E, Lagadec P, Imbert V, Peyron J-F, Rampal P, Nicoli JR, Czerucka D. 2010. Interaction of Saccharomyces
boulardii with Salmonella enterica Serovar Typhimurium Protects Mice and Modifies T84 Cell Response to the Infection. PLOS ONE 5:e8925.

122. Czerucka D, Dahan S, Mograbi B, Rossi B, Rampal P. 2000. Saccharomyces boulardii preserves the barrier function and modulates the signal transduction pathway induced in enteropathogenic Escherichia coli-infected T84 cells. Infection and immunity 68:5998-6004.

123. Castagliuolo I, LaMont JT, Nikulasson ST, Pothoulakis C. 1996. Saccharomyces boulardii protease inhibits Clostridium difficile toxin A effects in the rat ileum. Infection and immunity 64:5225-5232.

124. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. 1999. Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. Infection and immunity 67:302-307.

125. Brandão RL, Castro IM, Bambirra EA, Amaral SC, Fietto LG, Tropia MJ, Neves MJ, Dos Santos RG, Gomes NC, Nicoli JR. 1998. Intracellular signal triggered by cholera toxin in Saccharomyces boulardii and Saccharomyces cerevisiae. Applied and environmental microbiology 64:564-568.

126. Sheele J, Cartowski J, Dart A, Poddar A, Gupta S, Stashko E, Ravi BS, Nelson C, Gupta A. 2015. Saccharomyces boulardii and bismuth subsalicylate as low-cost interventions to reduce the duration and severity of cholera. Pathog Glob Health 109:275-82.

127. Gedek BR. 1999. Adherence of Escherichia coli serogroup O 157 and the Salmonella typhimurium mutant DT 104 to the surface of Saccharomyces boulardii. Mycoses 42:261-4.

128. Pontier-Bres R, Munro P, Boyer L, Anty R, Imbert V, Terciolo C, André F, Rampal P, Lemichez E, Peyron J-F, Czerucka D. 2014. Saccharomyces boulardii modifies
Salmonella typhimurium traffic and host immune responses along the intestinal tract.

PloS one 9:e103069-e103069.

Tiago FCP, Martins FS, Souza ELS, Pimenta PFP, Araujo HRC, Castro IM, Brandão RL, Nicoli JR. 2012. Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by Saccharomyces probiotics. J Med Microbiol 61:1194-1207.

Sovran B, Planchais J, Jegou S, Straube M, Natividad JM, Agus A, Dupraz L, Glodt J, Da Costa G, Michel M-L, Langella P, Richard ML, Sokol H. 2018. Enterobacteriaceae are essential for the modulation of colitis severity by fungi. Microbiome 6:152.

Wagner RD, Pierson C, Warner T, Dohnalek M, Farmer J, Roberts L, Hilty M, Balish E. 1997. Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. Infect Immun 65:4165-72.

Hager CL, Isham N, Schrom KP, Chandra J, McCormick T, Miyagi M, Ghannoum MA. 2019. Effects of a Novel Probiotic Combination on Pathogenic Bacterial-Fungal Polymicrobial Biofilms. mBio 10:e00338-19.

Hoarau G, Mukherjee PK, Gower-Rousseau C, Hager C, Chandra J, Retuerto MA, Neut C, Vermeire S, Clemente J, Colombel JF, Fujioka H, Poulain D, Sendid B, Ghannoum MA. 2016. Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn’s Disease. mBio 7:e01250-16.

Allonsius CN, van den Broek MFL, De Boeck I, Kiekens S, Oerlemans EFM, Kiekens F, Foubert K, Vandenheuvel D, Cos P, Delputte P, Lebeer S. 2017. Interplay between Lactobacillus rhamnosus GG and Candida and the involvement of exopolysaccharides. Microb Biotechnol 10:1753-1763.
135. Allonsius CN, Vandenheuvel D, Oerlemans EFM, Petrova MI, Donders GGG, Cos P, Delputte P, Lebeer S. 2019. Inhibition of Candida albicans morphogenesis by chitinase from Lactobacillus rhamnosus GG. Scientific Reports 9:2900.

136. Graham CE, Cruz MR, Garsin DA, Lorenz MC. 2017. Enterococcus faecalis bacteriocin EntV inhibits hyphal morphogenesis, biofilm formation, and virulence of Candida albicans. Proceedings of the National Academy of Sciences 114:4507.

137. Cabral DJ, Penumutchu S, Norris C, Morones-Ramirez JR, Belenky P. 2018. Microbial competition between Escherichia coli and Candida albicans reveals a soluble fungicidal factor. Microbial cell (Graz, Austria) 5:249-255.

138. Bandara HMHN, Cheung BPK, Watt RM, Jin LJ, Samaranayake LP. 2013. Secretory products of Escherichia coli biofilm modulate Candida biofilm formation and hyphal development. Journal of Investigative and Clinical Dentistry 4:186-199.

139. García C, Tebbji F, Daigneault M, Liu N-N, Köhler JR, Allen-Vercoe E, Sellam A. 2017. The Human Gut Microbial Metabolome Modulates Fungal Growth via the TOR Signaling Pathway. mSphere 2:e00555-17.

140. Valentine M, Benadé E, Mouton M, Khan W, Botha A. 2019. Binary interactions between the yeast Candida albicans and two gut-associated Bacteroides species. Microbial Pathogenesis 135:103619.

141. Yang W, Zhou Y, Wu C, Tang J. 2016. Enterohemorrhagic Escherichia coli promotes the invasion and tissue damage of enterocytes infected with Candida albicans in vitro. Sci Rep 6:37485.

142. Klaerner HG, Uknis ME, Acton RD, Dahlberg PS, Carlone-Jambor C, Dunn DL. 1997. Candida albicans and Escherichia coli Are Synergistic Pathogens during Experimental Microbial Peritonitis. Journal of Surgical Research 70:161-165.
143. Centeno A, Davis CP, Cohen MS, Warren MM. 1983. Modulation of Candida albicans attachment to human epithelial cells by bacteria and carbohydrates. Infection and immunity 39:1354-1360.

144. van Leeuwen PT, van der Peet JM, Bikker FJ, Hoogenkamp MA, Oliveira Paiva AM, Kostidis S, Mayboroda OA, Smits WK, Krom BP. 2016. Interspecies Interactions between Clostridium difficile and Candida albicans. mSphere 1:e00187-16.

145. Lambooij JM, Hoogenkamp MA, Brandt BW, Janus MM, Krom BP. 2017. Fungal mitochondrial oxygen consumption induces the growth of strict anaerobic bacteria. Fungal Genet Biol 109:1-6.

146. Panpetch W, Somboonna N, Palasuk M, Hiengrach P, Finkelman M, Tumwasorn S, Leelahavanichkul A. 2019. Oral Candida administration in a Clostridium difficile mouse model worsens disease severity but is attenuated by Bifidobacterium. PLoS One 14:e0210798.

147. Markey L, Shaban L, Green ER, Lemon KP, Mecsas J, Kumamoto CA. 2018. Pre-colonization with the commensal fungus Candida albicans reduces murine susceptibility to Clostridium difficile infection. Gut Microbes 9:497-509.

148. Siavoshi F, Heydari S, Shafiee M, Ahmadi S, Saniee P, Sarrafnejad A, Kolahdoozan S. 2019. Sequestration inside the yeast vacuole may enhance Helicobacter pylori survival against stressful condition. Infection, Genetics and Evolution 69:127-133.

149. Siavoshi F, Saniee P. 2014. Vacuoles of Candida yeast as a specialized niche for Helicobacter pylori. World J Gastroenterol 20:5263-73.

150. Sánchez-Alonzo K, Parra-Sepúlveda C, Vega S, Bernasconi H, Campos VL, Smith CT, Sáez K, García-Cancino A. 2020. In Vitro Incorporation of Helicobacter pylori into Candida albicans Caused by Acidic pH Stress. Pathogens (Basel, Switzerland) 9:489.
151. Mason KL, Erb Downward JR, Falkowski NR, Young VB, Kao JY, Huffnagle GB. 2012. Interplay between the gastric bacterial microbiota and Candida albicans during postantibiotic recolonization and gastritis. Infection and immunity 80:150-158.

152. Smith MG, Des Etages SG, Snyder M. 2004. Microbial synergy via an ethanol-triggered pathway. Mol Cell Biol 24:3874-84.

153. Trunk K, Peltier J, Liu Y-C, Dill BD, Walker L, Gow NAR, Stark MJR, Quinn J, Strahl H, Trost M, Coulthurst SJ. 2018. The type VI secretion system deploys antifungal effectors against microbial competitors. Nature Microbiology 3:920-931.

154. Tampakakis E, Peleg AY, Mylonakis E. 2009. Interaction of Candida albicans with an intestinal pathogen, Salmonella enterica serovar Typhimurium. Eukaryotic cell 8:732-737.

155. Kim Y, Mylonakis E. 2011. Killing of Candida albicans filaments by Salmonella enterica serovar Typhimurium is mediated by sopB effectors, parts of a type III secretion system. Eukaryotic cell 10:782-790.

156. Gaddy JA, Tomaras AP, Actis LA. 2009. The Acinetobacter baumannii 19606 OmpA Protein Plays a Role in Biofilm Formation on Abiotic Surfaces and in the Interaction of This Pathogen with Eukaryotic Cells. Infection and Immunity 77:3150-3160.

157. Peleg AY, Tampakakis E, Fuchs BB, Eliopoulos GM, Moellering RC, Mylonakis E. 2008. Prokaryote–eukaryote interactions identified by using Caenorhabditis elegans. Proceedings of the National Academy of Sciences 105:14585.

158. Fan D, Coughlin LA, Neubauer MM, Kim J, Kim MS, Zhan X, Simms-Waldrip TR, Xie Y, Hooper LV, Koh AY. 2015. Activation of HIF-1α and LL-37 by commensal bacteria inhibits Candida albicans colonization. Nature medicine 21:808-814.
159. Forbes JD, Bernstein CN, Tremlett H, Van Domselaar G, Knox NC. 2018. A Fungal World: Could the Gut Mycobiome Be Involved in Neurological Disease? Front Microbiol 9:3249.

160. Iliev ID, Leonardi I. 2017. Fungal dysbiosis: immunity and interactions at mucosal barriers. Nat Rev Immunol 17:635-646.

161. Li XV, Leonardi I, Iliev ID. 2019. Gut Mycobiota in Immunity and Inflammatory Disease. Immunity 50:1365-1379.

162. Lionakis MS, Iliev ID, Hohl TM. 2017. Immunity against fungi. JCI Insight 2.

163. Richard ML, Lamas B, Liguori G, Hoffmann TW, Sokol H. 2015. Gut fungal microbiota: the Yin and Yang of inflammatory bowel disease. Inflamm Bowel Dis 21:656-65.

164. Rizzetto L, De Filippo C, Cavalieri D. 2014. Richness and diversity of mammalian fungal communities shape innate and adaptive immunity in health and disease. Eur J Immunol 44:3166-81.

165. Lai GC, Tan TG, Pavelka N. 2019. The mammalian mycobiome: A complex system in a dynamic relationship with the host. Wiley Interdiscip Rev Syst Biol Med 11:e1438.

166. Romani L. 2011. Immunity to fungal infections. Nat Rev Immunol 11:275-88.

167. Brown GD. 2011. Innate antifungal immunity: the key role of phagocytes. Annu Rev Immunol 29:1-21.

168. Hoving JC, Wilson GJ, Brown GD. 2014. Signalling C-type lectin receptors, microbial recognition and immunity. Cell Microbiol 16:185-94.

169. Patin EC, Thompson A, Orr SJ. 2019. Pattern recognition receptors in fungal immunity. Semin Cell Dev Biol 89:24-33.
170. Ifrim DC, Bain JM, Reid DM, Oosting M, Verschueren I, Gow NA, van Krieken JH, Brown GD, Kullberg BJ, Joosten LA, van der Meer JW, Koentgen F, Erwig LP, Quintin J, Netea MG. 2014. Role of Dectin-2 for host defense against systemic infection with Candida glabrata. Infect Immun 82:1064-73.

171. Netea MG, Van Der Graaf CA, Vonk AG, Verschueren I, Van Der Meer JW, Kullberg BJ. 2002. The role of toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis. J Infect Dis 185:1483-9.

172. Stappers MHT, Clark AE, Aimanianda V, Bidula S, Reid DM, Asamaphan P, Hardison SE, Dambuza IM, Valsecchi I, Kerscher B, Plato A, Wallace CA, Yuecel R, Hebecker B, da Glória Teixeira Sousa M, Cunha C, Liu Y, Feizi T, Brakhage AA, Kwon-Chung KJ, Gow NAR, Zanda M, Piras M, Zanato C, Jaeger M, Netea MG, van de Veerdonk FL, Lacerda JF, Campos A, Carvalho A, Willment JA, Latgé JP, Brown GD. 2018. Recognition of DHN-melanin by a C-type lectin receptor is required for immunity to Aspergillus. Nature 555:382-386.

173. Jaeger M, van der Lee R, Cheng SC, Johnson MD, Kumar V, Ng A, Plantinga TS, Smeekens SP, Oosting M, Wang X, Barchet W, Fitzgerald K, Joosten LAB, Perfect JR, Wijmenga C, van de Veerdonk FL, Huynen MA, Xavier RJ, Kullberg BJ, Netea MG. 2015. The RIG-I-like helicase receptor MDA5 (IFIH1) is involved in the host defense against Candida infections. Eur J Clin Microbiol Infect Dis 34:963-974.

174. Salazar F, Brown GD. 2018. Antifungal Innate Immunity: A Perspective from the Last 10 Years. J Innate Immun 10:373-397.

175. van Tilburg Bernardes E, Pettersen VK, Gutierrez MW, Laforest-Lapointe I, Jendzjowsky NG, Cavin JB, Vicentini FA, Keenan CM, Ramay HR, Samara J, MacNaughton WK, Wilson RJA, Kelly MM, McCoy KD, Sharkey KA, Arrieta MC. 2020. Intestinal fungi are
causally implicated in microbiome assembly and immune development in mice. Nat Commun 11:2577.

Sokol H, Leducq V, Aschard H, Pham HP, Jegou S, Landman C, Cohen D, Liguori G, Bourrier A, Nion-Larmurier I, Cosnes J, Seksik P, Langella P, Skurnik D, Richard ML, Beaugerie L. 2017. Fungal microbiota dysbiosis in IBD. Gut 66:1039-1048.

Panpetch W, Hiengrach P, Nilgate S, Tumwasorn S, Somboonna N, Wilantho A, Chatthanathon P, Prueksapanich P, Leelahavanichkul A. 2020. Additional Candida albicans administration enhances the severity of dextran sulfate solution induced colitis mouse model through leaky gut-enhanced systemic inflammation and gut-dysbiosis but attenuated by Lactobacillus rhamnosus L34. Gut Microbes 11:465-480.

Wolf AJ, Limon JJ, Nguyen C, Prince A, Castro A, Underhill DM. 2020. Malassezia spp. induce inflammatory cytokines and activate NLRP3 inflammasomes in phagocytes. J Leukoc Biol doi:10.1002/jlb.2ma0820-259r.

Bacher P, Hohnstein T, Beerbaum E, Röcker M, Blango MG, Kaufmann S, Röhmel J, Eschenhagen P, Grehn C, Seidel K, Rickerts V, Lozza L, Stervbo U, Nienen M, Babel N, Milleck J, Assenmacher M, Cornely OA, Ziegler M, Wisplinghoff H, Heine G, Worm M, Siegmund B, Maul J, Creutz P, Tabeling C, Ruwwe-Glösenkamp C, Sander LE, Knosalla C, Brunke S, Hube B, Kniemeyer O, Brakhage AA, Schwarz C, Scheffold A. 2019. Human Antifungal Th17 Immunity and Pathology Rely on Cross-Reactivity against Candida albicans. Cell 176:1340-1355.e15.

Diehl GE, Longman RS, Zhang JX, Breart B, Galan C, Cuesta A, Schwab SR, Littman DR. 2013. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. Nature 494:116-20.
181. Leonardi I, Li X, Semon A, Li D, Doron I, Putzel G, Bar A, Prieto D, Rescigno M, McGovern DPB, Pla J, Iliev ID. 2018. CX3CR1(+) mononuclear phagocytes control immunity to intestinal fungi. Science 359:232-236.

182. Li X, Leonardi I, Semon A, Doron I, Gao IH, Putzel GG, Kim Y, Kabata H, Artis D, Fiers WD, Ramer-Tait AE, Iliev ID. 2018. Response to Fungal Dysbiosis by Gut-Resident CX3CR1(+) Mononuclear Phagocytes Aggravates Allergic Airway Disease. Cell Host Microbe 24:847-856.e4.

183. Break TJ, Oikonomou V, Dutzan N, Desai JV, Swidersgall M, Freiwald T, Chauss D, Harrison OJ, Alejo J, Williams DW, Pittaluga S, Lee CR, Bouladoux N, Swamydas M, Hoffman KW, Greenwell-Wild T, Bruno VM, Rosen LB, Lwin W, Renteria A, Pontejo SM, Shannon JP, Myles IA, Olbrich P, Ferré EMN, Schmitt M, Martin D, Barber DL, Solis NV, Notarangelo LD, Serreze DV, Matsumoto M, Hickman HD, Murphy PM, Anderson MS, Lim JK, Holland SM, Filler SG, Afzali B, Belkaid Y, Moutsopoulos NM, Lionakis MS. 2021. Aberrant type 1 immunity drives susceptibility to mucosal fungal infections. Science 371.

184. Mukherjee PK, Chandra J, Retuerto M, Sikaroodi M, Brown RE, Jurevic R, Salata RA, Lederman MM, Gillevet PM, Ghanoun MA. 2014. Oral mycobiome analysis of HIV-infected patients: identification of *Pichia* as an antagonist of opportunistic fungi. PLoS Pathog 10:e1003996.

185. Drell T, Lillsaar T, Tummeleht L, Simm J, Aaspöllu A, Väin E, Saarma I, Salumets A, Donders GG, Metsis M. 2013. Characterization of the vaginal micro- and mycobiome in asymptomatic reproductive-age Estonian women. PLoS One 8:e54379.

186. Nguyen LD, Viscogliosi E, Delhaes L. 2015. The lung mycobiome: an emerging field of the human respiratory microbiome. Front Microbiol 6:89.
van Woerden HC, Gregory C, Brown R, Marchesi JR, Hoogendoorn B, Matthews IP. 2013. Differences in fungi present in induced sputum samples from asthma patients and non-atopic controls: a community based case control study. BMC Infect Dis 13:69.

Hoffmann C, Dollive S, Grunberg S, Chen J, Li H, Wu GD, Lewis JD, Bushman FD. 2013. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. PLoS One 8:e66019.

Strati F, Di Paola M, Stefanini I, Albanese D, Rizzetto L, Lionetti P, Calabrò A, Jousson O, Donati C, Cavalieri D, De Filippo C. 2016. Age and Gender Affect the Composition of Fungal Population of the Human Gastrointestinal Tract. Front Microbiol 7:1227.

Goren I, Godny L, Reshef L, Yanai H, Gophna U, Tulchinsky H, Dotan I. 2019. Starch Consumption May Modify Antiglycan Antibodies and Fecal Fungal Composition in Patients With Ileo-Anal Pouch. Inflamm Bowel Dis 25:742-749.

**FIGURE LEGENDS**

**Figure 1:** Fungal populations in and on different anatomical sites

Fungal populations have been identified in and on almost all human body sites. This figure is a schematic representation of the most commonly identified fungal genera under non-pathological conditions in the oral cavity (53, 54, 56, 57, 184), skin (92, 103), urinary tract (6, 7), vagina (4, 185), breast milk (93), lungs (186, 187), and intestine (44, 96, 104, 188-190).

**Figure 2:** Specific fungal-bacterial interactions in the gastrointestinal tract
Schematic representation of the bacteria-fungi interactions discussed in this review. Localization in the cartoon is not a representation of where the interactions occur within a specific organ. Fungi are depicted outside of the organs for schematic purposes.
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Jason Devlin received his B.S. degree in biology from Benedictine University in 2017. He is currently a graduate research assistant in the laboratory of Dr. Judith Behnsen at the University of Illinois at Chicago, where he is working towards a Ph.D. in microbiology and immunology. His research interests involve understanding the interactions between pathogens, the microbiota, and the host and their contributions to infection. His current research investigates the roles of *Salmonella Typhimurium* chitinases during gastrointestinal infection.
