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Fungi in the healthy human gastrointestinal tract

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Abstract

Many species of fungi have been detected in the healthy human gut; however, nearly half of all taxa reported have only been found in one sample or one study. Fungi capable of growing in and colonizing the gut are limited to a small number of species, mostly *Candida* yeasts and yeasts in the family Dipodascaceae (*Galactomyces*, *Geotrichum*, *Saprochaete*). *Malassezia* and the filamentous fungus *Cladosporium* are potential colonizers; more work is needed to clarify their role. Other commonly-detected fungi come from the diet or environment but either cannot or do not colonize (*Penicillium* and *Debaryomyces* species, which are common on fermented foods but cannot grow at human body temperature), while still others have dietary or environmental sources (Saccharomyces cerevisiae, a fermentation agent and sometime probiotic; Aspergillus species, ubiquitous molds) yet are likely to impact gut ecology. The gut mycobiome appears less stable than the bacterial microbiome, and is likely subject to environmental factors.

Keywords: *Candida*, *Cladosporium*, dietary fungi; Dipodascaceae, gut fungal ecology, gut mycobiome, gut mycobiota, *Malassezia*, yeasts

Introduction

The gastrointestinal tract of an animal provides an attractive niche for organisms which have evolved to withstand its unique challenges. Nutrients ingested by the host are in steady supply and obviate the need to seek food. In the intestines, the outer mucus layer allows colonization and provides a nutrient rich habitat, while the dense inner layer segregates colonizing microbes from the dense concentration of immune cells in the intestinal epithelium.[1] To take advantage of these resources, organisms must be equipped to tolerate gut conditions: absence of oxygen; physiological temperatures (in mammals); peristaltic contractions and consequent movement of GI contents; variable pH, from the highly acidic stomach to the alkaline intestinal mucosa.

For most years since the development and acceptance of the germ theory, studies of human-associated microbes focused on pathogens.[2] While the existence of commensal and mutualistic relationships between humans and their gut microbiota has been known for well over a century (see, e.g., refs[3,4]), culture-based methods precluded study of any but the most tractable organisms. The development of culture-independent PCR-based methods revolutionized the study of the whole, healthy, human microbiome.[2] At the turn of the century, several large scale international healthy human microbiome projects were initiated.[5-7] With the decrease in cost and rise in capacity of next-generation sequencing technologies, microbiome projects are now more feasible to the single investigator. The contribution of the microbiome to overall well-being is now widely accepted and studied.

The healthy human gut microbiome contains members of all domains of life, with Eukarya primarily represented by the fungi and, in some populations, protists, notably *Blastocystis*.[8,9] The fungal component—the mycobiome—has received little attention compared with bacteria, but steady work by a number of researchers has produced a mature discipline, as evidenced by this special issue. Several important reviews have been published in this decade. Specifically, whole-body perspectives of the human mycobiome are provided by Cui and colleagues, [10] Huffnagle and Noverr,[11] Seed,[12] and Underhill and Iliev,[13] while the gut mycobiota is reviewed by Ianiro and colleagues,[14] Kirschner and colleagues,[15] and Suhr and Hallen-Adams.[16] The role of the gut mycobiota in disease is reviewed by Moyes and Naglik,[17] Wang and colleagues,[18] Gouba and Drancourt,[19] Mukherjee and colleagues,[20] and Richard and colleagues.[21] In this paper, we focus on the gut mycobiome of healthy humans, with a particular emphasis on the relatively few fungi that are widely distributed in human gut samples. We will also discuss the contributions of diet and the environment to gut fungal composition, and the stability of the gut mycobiome over time.

Many fungi have been reported from the human gut, but few are common. From 36 studies spanning 1917 to 2015 and using a broad array of culture-based and nonculture- based methods, 267 distinct, valid species were detailed.[16] (Taxa identified only to genus, or as "undescribed," in the studies were not included in this tally.) Two hundred species, or nearly 75%, were only reported in one study. A further 37 species were reported in 2 studies, and only 15 were reported in 5 or more studies. When studies involve multiple samples a similar trend is observed: many species may be observed, but a majority are only detected in a single sample. The studies from our own lab identified 97 distinct fungal taxa in 85 samples from 60 subjects.[22,23] Forty-eight taxa were limited to a single sample, while 14 occurred in 10 samples or more. The most commonly reported genera and species of gut fungi are given in Table 1. Most rarely detected taxa are unlikely to play a role in gut ecology or host health, for reasons discussed below.

Table 1. Most commonly-detected fungi in gut mycobiome studies.

Taxon[a]	# studies (%)[b]	# samples (%)[c]	References[d]
Candida	**32 (86%)**	**68 (80%)**	
C. albicans	26 (70%)	18 (21%)	3, 9, 22, 23, 31-33, 41-44, 46, 54-57, 59, 60-63, 65, 66, 68, 69
C. tropicalis	17 (46%)	57 (67%)	3, 22, 23, 29, 30- 33, 42, 44, 51, 54, 55, 60, 61, 62, 68
C. parapsilosis	13 (35%)	2 (2%)	9, 22, 30-33, 42, 55, 60, 61, 63, 67, 68, 69
C. glabrata	12 (32%)	0	3, 31, 41, 43, 44, 46, 54, 55, 59, 60, 61, 63
C. krusei	10 (27%)	0	3, 23, 31-33, 44, 45, 54, 55, 60, 63
C. lusitaniae	6 (16%)	0	30, 33, 43, 54, 55, 60
Saccharomyces	**20 (54%)**	**5 (6%)**	
S. cerevisiae	20 (54%)	5 (6%)	9, 22, 23, 30, 33, 34, 41-45, 51, 54, 58, 59, 61-64, 69
Penicillium	**14 (38%)**	**17 (20%)**	**9, 22, 42, 69d**
P. aff. *commune*	10 (27%)	10 (12%)	23, 29, 31, 41, 43, 44, 51, 58, 62, 67
Aspergillus	**12 (32%)**	**20 (24%)**	**22, 23, 42, 43, 51, 58, 62**
A. aff. *versicolor*	5 (14%)	0	9, 29, 31, 44, 67
Cryptococcus	**10 (27%)**	**3 (4%)**	**3, 22, 23, 30, 31, 41, 42, 60-62**
Malassezia	**11 (30%)**	**21 (25%)**	**69**
M. globosa	8 (22%)	1 (1%)	23, 29, 30, 34, 42, 43, 45, 58
M. restricta	7 (19%)	20 (24%)	22, 23, 29, 30, 43, 45, 58
M. pachydermatis	6 (16%)	1 (1%)	23, 29, 43, 44, 45, 58
Cladosporium	**10 (27%)**	**15 (18%)**	
C. aff. *herbarum*	10 (27%)	18 (21%)	22, 23, 30, 31, 32, 41, 43, 58, 61, 67
Galactomyces	**8 (22%)**	**8 (9%)**	**60**
G. geotrichum	7 (19%)	7 (8%)	9, 22, 29, 41, 43, 44, 45
Debaryomyces	**8 (22%)**	**18 (21%)**	
D. hansenii	7 (19%)	18 (21%)	22, 23, 30, 34, 43, 54, 59
Trichosporon	**6 (17%)**	**8 (9%)**	**22, 30, 33, 41, 43, 45**

a. Bold type refers to the genus as a whole; italics indicates individual species.
b. Based on 37 papers giving species-level identifications, published between 1917–2016.
c. Based on 85 samples published in refs. 22 & 23.
d. References for genus are only given if they are distinct from species references for that genus.

Categories of gut fungi

Fungi detected in the human gut can be split into resident and non-resident. As a minimum requirement, a resident (or autochthonous) fungus must be able to grow at 37_C to colonize the gut. For a few species of the wide and diverse yeast genus *Candida*, the mammalian digestive tract can be considered the primary niche. Species including *Candida albicans, C. tropicalis, C. parapsilosis*, and *C. glabrata* may all be found as natural, asymptomatic components of the human microbiome, and published estimates of *C. albicans* carriage in healthy individuals range from 30–60%.[24] Furthermore, many of these species do not appear to have a niche apart from living mammals; absent a source of contamination, they will not be found in significant concentrations in the air, or in soil, or in food.[25,26] *Malassezia* is another genus of yeasts whose primary niche is the mammalian microbiome; in fact, *Malassezia* species have lost the ability to synthesize their own lipids, so they are dependent on a host for their nutritional needs.[27] The known niche for *Malassezia* is skin, where it is the predominant fungal genus detected in 11 of 14 body sites (the exceptions all being on the foot; ref.[28]). *Malassezia* has also been reported in significant abundance in fecal samples and may play a role in the gut.[29,30,23] To determine whether any *Malassezia* is indigenous to the gut would require more invasive sampling techniques than the standard fecal collection, to eliminate the possibility of inoculation of the feces by skin flora.

Two further groups of fungi reported repeatedly in gut fungal studies likely do not have the gut as their primary niche but may be considered potential colonizers: *Cladosporium*, and yeasts in the Dipodascaceae (includes *Geotrichum/Saprochaete* and *Galactomyces*). *Cladosporium*, along with *Aspergillus* and *Penicillium* (discussed below) is a filamentous fungus, or mold; the other fungi commonly detected in the gut are all yeasts. *Cladosporium* has been reported in the healthy human GI tract (ileum as well as fecal samples) since 1969,[31] and was widespread in the microbiota of Apollo astronauts.[32] In the Dipodascaceae, yeasts in the genus *Galactomyces* have been reported in 22% of gut fungal studies, with *Galactomyces geotrichum* reported in 19% (see Table 1). A species related, but not identical, to *Geotrichum gigas* in the Dipodascaceae was detected in 54% of 69 samples by Hallen-Adams and colleagues,[22] but has only been reported from this study. *Cladosporium* and Dipodascaceae yeasts most likely enter the gut from environmental sources, but their occurrence is sufficiently common that some degree of colonization cannot be ruled out.

Saccharomyces cerevisiae – bakers' and brewers' yeast – in the gut presumably originates in food.[33,34] We would not consider *S. cerevisiae* an autochthonous gut organism nor a true human commensal (although it is often called commensal in the literature); it is a domesticated species of fermentations whose "wild" niche is associated with plants. However, the ability of strains to grow at 37°C and the opportunities for repeated introduction render it among the most commonly detected fungi in fecal samples, and it likely contributes to gut microbial ecology. Probiotic *S. cerevisiae* ("*S. boulardii*") does not persist for more than 5 d after administration stops in healthy subjects,[35] but has given rise to an increasing incidence of *Saccharomyces* fungemia when co-morbidities are present.[36] Many *Aspergillus* species fall into a similar category – they survive human physiological temperatures, but are much more commonly reported in environmental (soil, air, plant matter) than in gut samples, and are presumably of environmental origin.[37,38] Like *S. cerevisiae*, the potential for these species to persist and respond to the gut environment (see below) means they may play a role in the gut, regardless of preferred niche.

Other commonly-detected fungi include *Debaryomyces hansenii* and *Penicillium* aff. *commune*, foodborne species incapable of growing at 37 °C.[39,40] Finally, there is the long tail of rarely-detected fungi which make up most of the species richness in gut mycobiota studies but whose presence in human fecal samples is strictly incidental and cannot be assumed to influence the gut ecology. These include edible mushrooms,[23] plant pathogens (widely reported; see, e.g. refs.[41-43]), xerophiles (*Wallemia sebi* and *W. muriae*),[44] wood decay fungi,[22,45,46] and other organisms whose growth and nutrition requirements preclude any lasting role in the mammalian GI tract.

Influence of diet on gut fungi

The gut microbiome has been presumed sterile until birth, although detection of bacteria in amniotic fluid and in the meconium have raised recent challenges to that assumption.[47] Starting at birth and throughout life, the primary route for microbes to enter the gastrointestinal tract is via ingestion,[48] although inhalation can play a role as well. The first fungi detected in the infant gut are Saccharomycetalean yeasts, especially *Candida* species; these are presumed to be transmitted by the mother as *Candida* species are common inhabitants of the skin and vaginal mucosa as well as the colon.[49]

Providing both a means of entrance for microorganisms to the gut, and a major source of nutrients for established microbes, the diet is an obvious influence on gut microbial composition. David and colleagues found broad, reproducible, dietary-induced changes in the gut microbiome depending on whether volunteers consumed a plant- or an animal-based diet.[34] While bacterial composition showed a clear response to nutrient availability (carbohydrates/fiber vs. proteins and fats), fungal composition appeared to be driven by food colonization. Notably, the same species of fungi were detected in participant fecal samples and in cheese fed to those participants. [34] Hoffmann and colleagues found that *Candida* abundance was positively correlated with recent consumption of carbohydrates and negatively correlated with total saturated fatty acids.[50] Recent consumption of short chain fatty acids drove down the abundance of *Aspergillus*.[50] Ukhanova et al. found a decrease in *Candida* and *Penicillium* related to almond and pistachio consumption.[51]

Finally, Suhr and colleagues examined 16 samples from 15 vegetarians, while Hallen-Adams et al. used the same methodology in the same laboratory to isolate and sequence fungal DNA from 69 samples from 45 people on a conventional Western diet.[22,23] The distribution of fungi differed considerably between the 2 groups (Table 2). Plant pathogenic *Fusarium* was detected in all but 2 samples from vegetarians (14 samples; 88%), while it was only detected in 2 samples from participants on conventional diets (3%). *Malassezia* and (presumed) foodborne *Penicillium* and *Aspergillus* were also present in more than 50% of vegetarian samples but much rarer in conventional diet samples. Common fungi were also proportionally more common in vegetarian samples; the top 5 genera were detected in 88, 81, 75, 68 and 63% of samples, respectively, while the top 5 genera in conventional diet samples were detected in 84, 46, 16, 16 and 12%. These differences are not due solely to the smaller sample set for vegetarians; *Fusarium, Malassezia, Penicillium* and *Aspergillus* were detected in a higher number of vegetarian than conventional diet samples. Conversely, David and colleagues found a significant enrichment of *Penicillium* in participants on an animal-based diet than on

Table 2. Most common taxa in vegetarian and conventional diet samples.

Genus	Vegetarian	Conventional
Fusarium	14 (88%)	2 (3%)
Candida	10 (63%)	58 (84%)
Malassezia	13 (81%)	8 (12%)
Penicillium	12 (75%)	1 (1%)
Aspergillus	11 (68%)	4 (6%)
Geotrichum	ND	32 (46%)
Pichia	1 (6%)	11 (16%)
Cladosporium	4 (25%)	11 (16%)

Data from refs. 22 & 23.

a plant-based diet (and an overall enrichment in fungal transcripts and CFUs on the animal-based diet);[34] however, their controlled animal-based diet was rich in cheeses, including Camembert and blue which are processed with *Penicillium* and were absent from the plant-based diet. The vegetarian subjects in Suhr et al.[22] included lacto-ovo vegetarians, who consume dairy.

Studies to date provide intriguing hints about the role of diet in influencing the gut mycobiome, but broad conclusions are precluded by the limited number of studies and the differing methodologies. To date, only David et al. have conducted a controlled diet study;[34] their findings were sufficiently dramatic to demonstrate the benefit of such studies. Additional studies, involving multiple locations and populations and incorporating detailed dietary information, would be valuable in clarifying the impact of diet on fungi. Environmental sampling for studies taking place in circumscribed environments, such as hospitals, and sampling of participants' skin and oral mycobiome, could suggest other sources of gut fungi.

Stability of the gut mycobiome

While the stability of the bacterial microbiome is now well-documented (e.g., ref.[52]), the situation in fungi is less clear. Comparatively few studies have genus- or species- level fungal data for multiple samples from the same subject over time, and those who have addressed the issue have reached differing conclusions. Our own impression, based on samples at 2 time points each from 24 participants, is that the composition of the gut mycobiome is not particularly stable; we found the same fungus at both time points less than 20% of the time.[22] Given the high proportion of rare, incidental taxa detected in fecal samples, we eliminated these taxa from consideration, which led to a slight increase; commonly-detected gut fungi were detected in both time points 27% of the time. In this dataset only the most common fungus, *Candida tropicalis*, was present at both time points a majority of the time (58%).

Mouse studies have shown variability over time,[53] leading Underhill and Iliev to generalize that "(t)his suggests that commensal fungal populations are more variable than those of bacteria and that they may be influenced by fungi in the environment."[13] Anderson, writing near the advent of gut mycobiota studies observed "(t)he blastomycetes [= yeasts] must, for the present be regarded as accidental ingredients of the feces, since in the same case observed at different periods they vary so extraordinarily"[3] Possibly in contradiction of the foregoing, Cohen and colleagues report "(t)he stability of the mycofloral pattern of the small intestine was demonstrated in 5 subjects who were resampled 5 to 9 months after the initial studies; the fungal pattern was qualitatively and quantitatively unchanged,"[31] however, these authors sampled from multiple parts of the GI tract and specified the small intestine in their observations, while other studies have been limited to the feces. More studies, with samples collected over multiple time points, are needed to address the matter of gut mycobiome persistence.

Concluding remarks

Multiple studies have now provided a baseline snapshot of the gut mycobiome in healthy individuals. While more studies and more samples would address some outstanding questions (are there geographical differences in mycobiome? Differences based on age, gender or other demographic factors?), we can begin to make some broad generalizations about which groups of fungi are likely to be of importance to the host and the overall microbiome. As far as possible, new studies should take diet and the environment into account, which can be as simple a matter as requesting a dietary log and including one or more relevant skin (hands and face may come into contact with food and contribute organisms which are then swallowed, while elbow or foot samples are not likely to) and oral sample from participants. Sequencing multiple samples over time from as large a pool of participants as possible would be highly desirable; at present, mycobiome studies lag far behind bacterial microbiome studies in terms of numbers and consequent statistical power.

Many unanswered questions remain about those fungi whose niche is the gut: what are they doing in a healthy host, and would their absence be detrimental? Are species interchangeable, i.e., is there any effect in replacing *Candida albicans* with *C. tropicalis* or *C. parapsilosis* in an individual host? Do species, or strains of the same species, compete and, if so, are there predictable outcomes? – Given the importance of *Candida* yeasts as opportunistic pathogens, answering these questions could provide insight into limiting or preventing yeast infections or candidiasis. Whether there are gut-resident strains or species of *Malassezia* or *Cladosporium* remains to be answered as well.

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