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GUT MYCOBIOME IN HEALTH AND DISEASE

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Abstract – The complexity of the human microbiota remains poorly understood, despite the evolutionary insights gained in recent years. In particular, the functional role of fungi as one of the key players in the human microbiota remains to be defined. This review provides a summary of the emerging data on the mycobiota in health and disease published in the last year. This work provides a structured insight into the involvement of fungi in liver disease, inflammatory bowel disease and cancer. The data presented support the critical role of fungi in human disease, and changes with mostly increased fungal abundance are associated with specific diseases. Future studies are needed to elucidate the functional role and interaction between the mycobiome and clinical phenotype, as well as mechanistic insights within the microbiota and host interaction.

Keywords: Mycobiome, Microbiota, Cancer, Health, Fungi, Liver, IBD.

INTRODUCTION

The involvement of the microbiota in health and disease is an emerging research topic and much evidence has been gathered on its important role. While there is a lot of data on bacteria in the healthy state[1], this issue has not yet been comprehensively addressed for fungi. In particular, mycobiota are considered to play a meaningful role in human diseases. Fungi are frequently considered pathogens, such as the yeast *Candida albicans*, which colonizes the oral cavity[2], vagina[3], and also the human intestine[4]. For example, *C. albicans* is one of the most common fungal pathogens that can cause life-treating infections. It is quite adaptable and is associated with the formation of biofilms in humans which is relevant for the development of resistance[5]. In this review, we provide an overview of the most urgent work published during the past year (from March 2022 to March 2023) on mycobiome and its influence on health and disease in general, on liver diseases, inflammatory bowel disease (IBD) and cancer in particular.

MYCOBIOME IN HEALTH AND DISEASE

The data on the impact of fungi in health and in disease are in the early stage of its development. A recent work by Shuai et al[6] investigated the stability of the mycobiome in 1,244 middle-aged and elderly people from the Guangzhou Nutrition and Health Study with a focus on aging and diet. The authors observed that with age, *Blastobotrys* and *Agricomycetes spp.* were depleted in favor of a higher abundance of *Malassezia spp.* Interestingly, there was an association be-

tween diet and fungal changes. Consumption of dairy products was associated with an increased abundance of *Saccharomyces*, whereas *Candida spp*. was decreased. In addition, the authors investigated the interaction between the gut fungal and bacterial communities and found an interplay between *Saccharomyces* spp. and the gut microbiome that improved insulin sensitivity. Moreover, gut bacteria and fecal histidine influenced cholesterol metabolism, especially *via* the regulation of LDL-blood levels[6].

Since an interplay between the host's genetics, diet, and the composition of the host's microbiome becomes more and more evident, a recent work from Gupta et al[7] analyzed the less-known role of genetics and diet in the composition of the host's fungal composition. In a murine model of an intercrossed mouse line and by using whole genome sequencing and genotyping, single genes could be linked to variations of the murine mycobiome. Moreover, the authors also found an altered composition of the mycobiome as well as the microbiome. So, the composition of the mycobiome depends on a complex interaction between the hosts' genetics, environmental factors like diet, and the microbiota[7]. In the mouse model, there is evidence for the interaction between the immune system and fungal-bacterial interaction. Leonardi et al[8] distinguished mucosa-associated fungi such as *C. albicans, S. cerevisiae and S. fibuligera* and lumen-associated fungi (*Aspergillus amstellodamii, Cladosporium cladosporioides* and *Wallemia sebi)*. In a murine model of antibiotic and DSS-treated mice, the mucosal fungi provided protection against intestinal injury *via* reduction of permeability and reduced mortality, while the lumen-associated fungi showed no such effect. With regard to the role of the immune system, mice treated with the mucosal mushrooms had higher levels of IL-22 and IL-17-producing T-helper cells. Further experiments with IL-22- and IL-17-deficient mice showed that IL-22 was the main reason for the protective effect. In contrast, social behavior was mediated by IL-17R-dependent signaling in neurons, suggesting a complex interaction between gut mycobiota and neuroimmune modulation in the gut-brain axis[8].

To further investigate the role of the mycobiome in cardiovascular disease and the immune system, Zou et al[9] used ITS1-ITS2 sequencing to examine the composition of the mycobiome in fecal samples from patients with hypertension, prehypertension, and normal blood pressure. In patients with normal blood pressure, *Mortierella* was enriched, whereas *Malassezia* was increased in patients with hypertension. In general, patients with hypertension and prehypertension had higher fungal diversity and abundance. The authors also reported a positive association between *Malassezia* and serum levels of light chains in patients with hypertension[9]. Using a similar approach, Chen et al[10] compared the oral and intestinal mycobiomes of patients with normal blood pressure and those with arterial hypertension. They found a higher abundance of *Exophiala spp*. in subjects with hypertension. *Exophiala xenobiotica* and *Exophiala mesophile* even correlated directly with the degree of hypertension. All in all, fungi were associated with the pathogenesis of hypertension[10]. However, further studies are needed to investigate possible causal links between the mycobiome and hypertension.

The more known gut-brain axis also affects diseases like multiple sclerosis (MS). Gargano et al[11] compared the mycobiome of healthy controls to patients with MS. The fungal diversity, especially *S. cerevisiae*, was higher in MS patients compared to the controls. Mechanistically, higher activation of mucosal-associated invariant T-cells (MAIT) caused by *Saccharomyces*, but also *Candida*, *via* IL-23 is discussed. Those MAIT are suspected to play an important role in autoimmune diseases like MS[11]. Furthermore, Liu et al[12] investigated the role of the vaginal/uterine mycobiome in patients with intrauterine adhesions (IUA) compared to healthy controls. In IUA patients, *Filobasidium* and *Exophiala* were enriched. Further investigation in a murine model showed an anti-inflammatory effect for *C. parasitosis via* interaction with the local bacteria[12]. In addition, Zhao et al[13] found differences in the vaginal microbiome in vulvovaginal candidiasis depending on whether *C. glabatra* or *C. albicans* were the cause of the disease. While *C. glabatra* was more associated with *Prevotella*, which is causing bacterial vaginosis, *C. albicans* was linked to *Ureaplasma urealyticum* infection[13].

Bao et al[14] investigated the relationship between fungi and type 2 diabetes mellitus (T2D) in mice and found a positive correlation between intestinal *C. albicans* and T2D. In a high-fat diet (HFD) model, eradication of the mycobiome improved insulin sensitivity in mice and prevented metabolic dysfunction. The reintroduction of *C. albicans* exacerbated insulin resistance and led to metabolic dysfunction. The authors hypothesized a b-glucan-induced activation of the dectin-1-dependent pathway as a potential causal mechanism[14]. Provokingly, Peroumal et al[15] per-

formed a comparable study with an opposite result in a long-term *C. albicans* feeding model. The mycobiome-microbiota interaction in mice was dependent on *C. albicans*, which also regulated appetite-regulating hormones and body weight[15]. Gutierrez et al[16] also looked at the effect of the mycobiome on BMI in infants. They found complex interactions depending on the microbiome/mycobiome interaction. *Saccharomyces* and *Malassezia* were positively associated with BMI in early life, while *Rhodotorula* was negatively associated[16]. Taken together, the role of the mycobiome in host metabolism is still unclear and requires further study. However, by influencing metabolism, the mycobiome is also likely to influence diseases that result from dysregulated metabolism.

Another aspect of ongoing research is the interplay in the "gut-lung" axis. Narayana et al[17] evaluated mycobial alterations in patients with bronchiectasis. They found that high similarity between the microbiome of the gut and the lung leads to more severe disease and exacerbations. Part of the fungal community was *Saccharomyces*. *Candida,* as a more frequent commensal in the lungs, correlates with less disease severity[17].

The focus of this review is the GI tract and liver diseases. The summary of the results is shown in Table 1 and schematically demonstrated in Figure 1.

MYCOBIOME IN LIVER DISEASES

An increasing number of studies focus on fungi and liver diseases in the "gut-liver axis"[18]. Demir et al[19] studied the faecal mycobiome of patients with NAFLD. They showed that non-obese patients with non-alcoholic steatohepatitis (NASH) or F2-F4 fibrosis differed significantly from non-obese patients with NAFLD or F0-F1 fibrosis. Based on a multinomial regression analysis, certain fungi could be assigned to disease. For example, *C. albicans, C. argentea* and *Penicillium* were associated with NASH. Also, certain *Saccharomycetales* and *Malassezia* were associated with NAFLD. The author showed that the ratio of *C. albicans* to *S. cerevisiae* correlates with transaminase levels and the histological degree of inflammation, while the *Mucor sp./S. cerevisiae* ratio correlates with blood glucose levels and is associated with an increased degree of fibrosis and inflammation. In addition, network analysis showed that *C. albicans* was associated with *Eubacterium hallii* and *Bifidobacterium adolescentis*. These results were reproduced in an FMT-mouse model (germ-free C57BL/6 mice) from NASH patients with and without antifungal drug amphotericin B. This group showed a better outcome in terms of liver weight/body weight ratio and lower ALT levels, as well as reduced hepatic triglyceride and cholesterol concentrations. In addition, amphotericin B was linked to reduced liver inflammation as indicated by lower levels of TNFα-mRNA. Mbaye et al[20] studied mycobiome in patients with NASH for endogenous alcohol and triglyceride production and compared them with a control group. They found increased levels of faecal alcohol in the NASH group, as well as a higher occurrence of fungi. *Pichia kudriavzevii* was present in 4 of the 10 patients. Other fungi included *C. glabrata, C. albicans* and *Galactomyces geotrichum*. Further *in vitro* analysis revealed that *P. kudriavzevii*, as well as *C. glabrata* and *C. albicans*, tended to produce more ethanol from fructose, while *Geotrichum candidum* tended to produce only minimal amounts. Overall, this study also showed that yeasts produce about 10 times more ethanol than bacteria[20].

The faecal mycobiome has also been studied in malignant primary liver tumours such as hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). Zhang et al[21] looked at the gut mycobiome in liver cirrhosis (LC) and HCC patients. Reduced alpha diversity was found in the LC group compared to the control cohort, while no change in alpha diversity was observed in the HCC group. An altered mycobiome with an increased abundance were found in the HCC group compared to the control group. The comparison between HCC and LC showed that *Saccharomyces*, but also *C. albicans* and *Malassezia* were increased in the HCC group and *Archaeorhizomycetes* in the LC group. Similar results can be seen for the ICC as well. Zhang et al[22] examined the gut mycobiome between a healthy control cohort and patients with ICC. They showed that the ICC patients had a significantly altered mycobiome and lower alpha diversity. Looking at the individual genera, similar to HCC, *C. albicans* was increased in the ICC group, whereas *S. cerevisiae* was increased in the control group. In addition, *C. albicans* was found to be more enriched at higher tumour stages.

TABLE 1. OVERVIEW OF PUBLICATIONS RELATED TO MYCOBIOME IN HEALTH AND DISEASE.

Topic	Authors	Ref.	Key points
Mycobiome in health and disease	Shuai et al	6	The composition of the mycobiome changes in the course of life and depends on diet
	Gupta et al	7	Host's Genetics influence the Mycobiome
	Leonardi et al	8	MUC increased IL-17 and IL-22 production and protect from intestinal injury
	Zou et al	9	*Malassezia* and fungal diversity is increased in patients with arterial hypertension
	Chen et al	10	*Exophiala spp.* correlate with hypertension
	Gargano et al	11	In MS patients the fungal diversity is increased and may act *via* MAIT activation
	Liu et al	12	*Filobasidium* and *Exophiala* are enriched in IUA whereas C. parapsilosis is anti-inflammatory
	Zhao et al	13	C. glabatra is associated with bacterial vaginosis whereas *C. albicans* favors *Ureaplasma urealyticum* infection
	Bao et al	14	*C. albicans* worsen insulin resistance in HFD and lead to metabolic disorders
	Peroumal et al	15	*C. albicans* regulates appetite and can also lead to a healthy body weight
	Gutierrez et al	16	Mycobiome/Microbiome interaction influences BMI in early life
	Narayama et al	17	A gut-similar microbiome in the lung is associated with a more severe disease in patients with bronchiectasis
Mycobiome in liver disease	Demir et al	19	The fecal mycobiome of non-obese patients with advanced NAFLD differs from that of non-obese patients with early NAFLD and administration of amphotericin B improves outcome
	Mbaye et al	20	In patients with NASH more ethanol mainly produced by fungi can be detected
	Zhang et al	21	The fecal mycobiome of HCC patients differs with an increase in *C. albicans* and a correlation to higher stages.
	Zhang et al	22	Patients with ICC show a reduced alpha diversity, and altered fecal mycobiome, in which *C. albicans* is increased and correlates with higher tumor stages
Mycobiome in IBD	Li et al	23	*C. albicans* strains were associated with intestinal inflammation in UC patients and IL-17A expression was influenced C. albicans
	Yu et al	24	IBD patients with CDI also vary in the mycobiome.
Mycobiome in Cancer	Narunsky-Haziza et al	25	Different tumour types had higher fungal abundance than healthy controls *C. albicans* and *S. cerevisiae* increase in colon cancer. *Malassezia* globose increase in breast cancer with survival decrease for patients
	Dohlman et al	26	Fungi are enriched in cancer tissue and *Candida* induces pro-inflammatory pathways.
	He et al	27	Fungal protein profiles differ in patients with OSCC
	Heng et al	28	Increasing fungi abundance in OSCC carcinoma with diagnostic potential.
	Yang et al	30	Specific fungal community in gastric cancer with diagnostic potential.
	Lin et al	31	*Aspergillus rambellii* was enriched in colon cancer and promoted colorectal tumorigenesis

MUC = mucosa-associated fungi, HFD = high fat diet, CKD = chronic kidney disease, MS = multiple sclerosis, MAIT = mucosal invariant T-cells, IUA = intrauterine adhesion, OSCC = oral squamous cell cancer, NAFLD = non-alcoholic fatty liver disease, NASH = non-alcoholic steatohepatitis, HCC = hepatocellular carcinoma, ICC = intrahepatic cholangiocellular carcinoma, IBD = inflammatory bowel disease, CDI = Clostridioides difficile infection.

MYCOBIOME IN IBD (GI-DISEASES)

Dysregulated host-microbiota interaction plays an important role in the pathogenesis of IBD, although the role of the mycobiome is still unclear. Li et al[23] found a large diversity of opportunistic

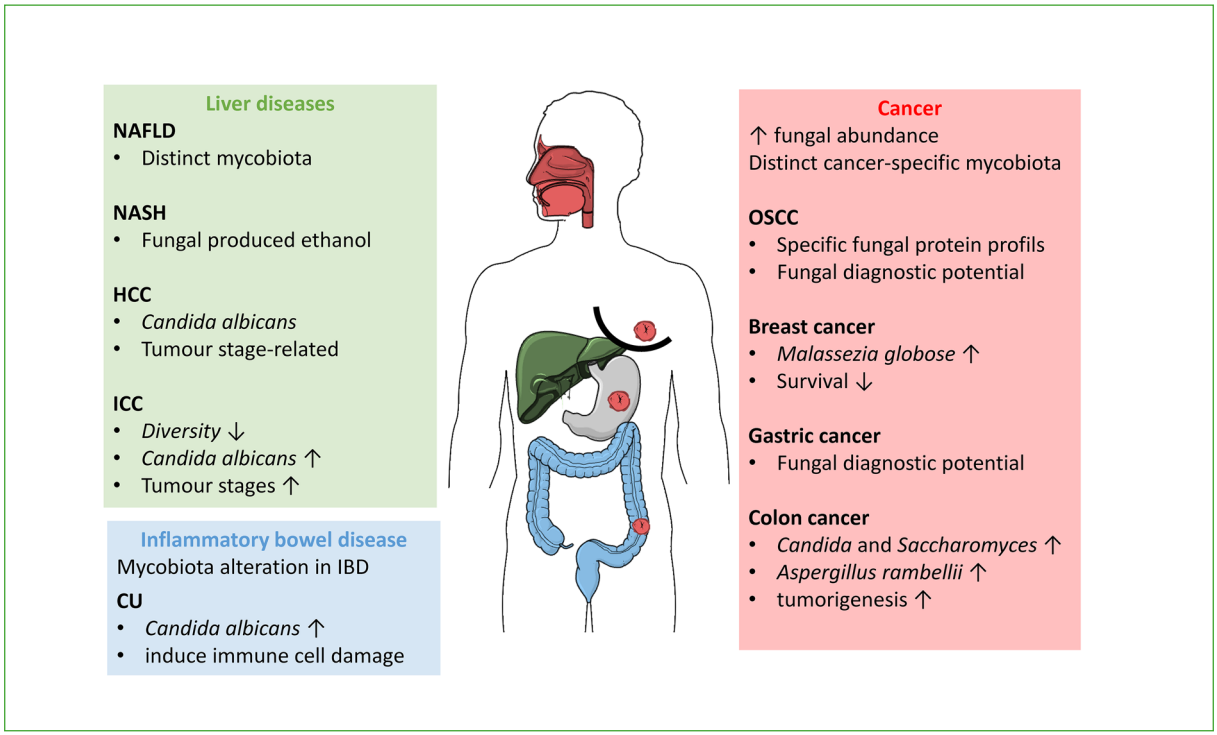

Figure 1. Summary of the data linking mycobiome and gastrointestinal tract including liver diseases, inflammatory bowel disease and gastrointestinal cancers.

C. albicans. Among them, strains with a high capacity to damage immune cells (summarized as HD strains) were associated with intestinal inflammation in UC patients in an IL-1b-dependent manner. The expression of another inflammatory cytokine, IL-17A, was influenced by lysin-expressing *C. albicans*, transforming this commensal fungus into a pathogenic strain in UC patients[23]. Yu et al[24] evaluated mycobiome in IBD patients with and without *Clostridioides difficile* infection (CDI). IBD patients with *C. difficile* infection had compared to those without *C. difficile* infection not only a higher abundance of *S. cerevisiae* and *C. albicans* but also as evidence for an increased peptidoglycan synthesis[24].

MYCOBIOME IN CANCER

While the role of the microbiome in cancer has been extensively studied, the role of the fungal community (mycobiome) has been widely neglected over the past years. One of the key publications of the past year is likely a pivotal study where the authors performed a comprehensive pan-cancer analysis of fungal ecologies and bacteriome interactions[25]. The study included 17,401 samples from tissues, blood and plasma across 35 cancer types (including breast, lung, melanoma, ovarian, colon, brain, bone, and pancreatic cancer) in 4 independent cohorts. According to the results, the fungal abundance was quantified to be higher in cancer than in healthy controls, but overall, the abundance was low and varied among tumour types. As previously reported for certain cancers, specific fungi species could also be detected directly in tumour tissue using b-glucan or *Aspergillus* staining and the clustering based on fungal sequences revealed a cancer-type specific mycobiota. The biostatistical functional analysis in network interaction with bacterial communities and immunomes gave evidence for the existence of so-called bi-domain ecologies in rather permissive than competitive microenvironments with distinct immune responses. The assessment for biomarker properties revealed prognostic and diagnostic capacities, which are reported in a cancer-specific manner. Tumour-associated mycobiomes were also deeply dissected by another pan-cancer analysis of multiple body sites by Dohlman et al[26], where the authors quantified the proportion of fungi to tumour cells as 1:10^4. Overall, the authors

linked *Blastomyces* to lung tumour tissues. Most interestingly, they observed a link between *Candida* to gastric cancer and colorectal cancer, implicating its role in pathogenesis as a diagnostic and prognostic biomarker.

He et al[27] analysed the mycobiota by accessing publicly available mass spectrometry data from oral squamous cell carcinoma (OSCC). 934 proteins belonging to 228 fungal species were identified in the data. Of these 934 proteins, 196 showed significant differences in abundance between OSCC and controls, and most of them were more highly expressed in OSCC patients. These expression differences confer discriminatory power to fugal proteins and may also have diagnostic potential for OSCC[27]. The importance of the oral microbiota for OSCC was also investigated using a sequencing approach. Heng et al[28] analysed the mycobiome and microbiome from different oral cavities (buccal mucosa, supragingival plaque, and saliva) in healthy controls and patients with premalignant oral lesions (OPL) and OSCC patients. Fungal species, such as *Acremonium exuviarum*, *Aspergillus fumigatus*, and *C. tropicalis*, were increased in the OSCC group, while others, such as the bacterial species *Streptococcus salivarius* subsp. *salivarius* or the fungal genus *Morchella* decreased in this group. *Streptococcus* and *Candida* also showed a negative correlation with each other. Based on the different fungal profiles, the authors evaluated the diagnostic value of the mycobiota with a receiver operating characteristic (ROC) curve, and the results showed discrimination only between HC and OSCC, but not between OLP and OSCC[28]. Overall, OSCC shows an altered fungal profile, but further studies are needed to clarify a possible mechanistic relevance.

The fungus *Malassezia* is associated with breast cancer, which was reported by Narunsky-Haziza et al[25] and Dohlman et al[26]. The results also showed that *M. globose* can significantly shorten the survival of breast cancer patients, demonstrating the clinical utility of cancer mycobiota[25].

The role of microbiota in gastric cancer (GC) is an emerging field with potential translational relevance as certain bacterial taxa, for instance, Fusobacterium nucleatum, have been recently linked with the prognosis of GC patients[29]. Yang et al[30] evaluated mycobiota in GC. The authors found that the fungal community of GC patients and healthy controls differed significantly. Fungi such as *Cutaneotrichosporon* and *Apiotrichum* were enriched in GC and showed diagnostic potential for GC, along with 8 other fungal genera[30]. In another study, high rates of *Candida* were linked to the expression of pro-inflammatory immune pathways in GC[26].

A high-quality study[31] evaluated microbiome and mycobiome in Colorectal cancer (CRC). The authors analysed multiple CRC cohorts using a metagenomic approach and found a strong correlation between *F. nucleatum* and the fungus *Aspergillus rambellii*. *A. rambellii* was enriched in CRC and promoted colorectal tumorigenesis *in vitro* and *in vivo* using a colon cancer organoid and xenograft models[31]. Several other studies also reported an altered mycobiota for CRC. For example, a higher abundance of *C. albicans* and *S. cerevisiae* in colon cancer[25] and *Candida* genus was predictive of metastatic disease and attenuated cellular adhesions[26].

CONCLUSIONS

In summary, the past year has seen a further layer of evidence linking the mycobiome to health and disease. In particular, it is clear that fungi interact closely with bacteria to form a microbial network. The excellent data presented in this review highlight the role of fungi in shaping disease development. Compared with the microbial community, the disease state is often associated with an increase in fungal abundance or a change in the structure of the mycobiota. Beyond associative evidence in translational studies, the causal role of fungi in mucosal or immune system deregulation is convincingly observed. While further studies are needed to unravel the role of fungi in all aspects of health and disease, there are other challenges beyond the much-needed mechanistic studies. Compared to the microbiome, where community analysis using 16s rRNA (e.g., V1-V2 region[32]) is well applied, the best technical modality to study fungi, which may be present at low abundance, remains to be determined. Fungi, like eukaryotes, also have introns in their DNA that are not translated into proteins [internal transcribed spacer (ITS)], which is commonly used for sequencing. However, the performance of different methods and primers can vary, making it difficult to compare studies[33]. Progress in fungal research is ongoing, and the scientific community will undoubtedly continue to be excited about filling in the gaps in our current knowledge in the coming year.

Conflict of Interest

AL received speaker fee from Janssen and advisory fee from Ferring. Other authors declare no potential conflicts of interest.

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Authors’ Contribution

All authors took part in writing, reviewing and editing of the manuscript. JL and RJ contributed equally to the work.

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