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Unveiling the mycobiota: The fungal frontier of human health

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A B S T R A C T

The microbiota and its effect on health has been extensively studied over the past decade. In many studies, the term microbiota has become synonymous with the bacterial component of the microbiota. Other microbes in the microbiota, such as viruses and fungi, have been neglected until recently. This special issue provides some background on the mycobiota and explores the role of gut fungi in human diseases such as cancer, metabolic diseases, and infection by *Clostridiodes difficile*, and describes the incidence of fungal infections in transplant patients. The mycobiota, once overlooked, now garners increasing attention.

1. Introduction

The Human Genome Project spearheaded understanding of the microbiota's role in human health and disease, catalyzing a deeper exploration of microbial communities inhabiting the body. While initial efforts concentrated on bacteria, a new microbial frontier has emerged: the mycobiota. The mycobiota's influence extends beyond the human body, as it interacts with environmental factors and other microbiotas, such as those found in the soil and air. This interconnectivity highlights the need for a holistic approach to studying the mycobiota, considering the complex interplay between fungi, bacteria, bacteriophages, and their respective environments [1].

Initially, the study of the mycobiota experienced many technological limitations [2]. While culture-based techniques failed to capture the full diversity of the mycobiota, molecular methods lacked universally accepted genetic markers and comprehensive databases akin to those for bacteria. The lack of specialized analytical tools, standardized methodologies, and the intrinsic variations in fungal biomass due to diet, oral hygiene, and environmental exposures compounded the challenges researchers faced in studying the mycobiota.

However, a surge in innovative technologies such as next-generation sequencing methods, and metagenomic and amplicon sequencing, have revolutionized our ability to identify and characterize fungal species in the human body. Culture-independent techniques such as quantitative PCR and fluorescence in situ hybridization have enabled the quantification and visualization of fungi in their native environments.

Even though fungal genomes represent a minuscule fraction of the total microbial genome, the secondary metabolites produced during interactions with bacteria and the host cells play a significant role in immune modulation and metabolic homeostasis [3].

1.1. Effect of the mycobiota on homeostasis

Residents of the mycobiota interact synergistically with each other through various mechanisms. *Candida* species metabolize complex carbohydrates and make them available to the bacteria *Prevotella* and *Ruminococcus*, which in turn release the fermented byproducts to *Methanobrevibacter* which, in turn, releases methane and carbon dioxide as the end products. This is an example of a syntropic guild [4], which refers to a community of microbes within an ecosystem that collaborate in a mutually beneficial manner.

1.2. Immune system modulation

Fungal commensals can modulate the immune system, its responses and tolerance, and maintain homeostasis. Gut fungal species prompt regulatory T cells (T_{reg} cells) to maintain immune tolerance to commensals. These commensals can activate the macrophages in the innate immune system to release pro-inflammatory cytokines [5–8]. Fungal antigens influence adaptive immunity by stimulating T-cell responses leading to the formation of memory T cells conferring long-term immunity to fungal infections. Beta-glucans from fungal cell walls interact with dectin-1 (a pattern recognition receptor in the immune system) to modulate and activate inflammatory responses.

A commensal fungal organism, *Saccharomyces boulardii*, produces a protease that deactivates toxins A and B produced by *Clostridium difficile*.

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The organism has been used as a probiotic against several other gastrointestinal pathogens such as *Escherichia coli*, *Helicobacter pylori*, *Vibrio cholerae*, and *Shigella flexneri* [9]. Probiotic formulas including *S. boulardii*, *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, and *Bifidobacterium breve* inhibit biofilms containing *E. coli* and *Candida* species.

1.3. Dysbiosis of the mycobiota

Dysbiosis of the mycobiota occurs due to aging, unhealthy diet, poor oral hygiene, use of antibiotics, differences in urban vs rural settings, exposure to soil, and other contaminants in the environment [10]. Consumption of highly processed food and drinks also reduces fungal diversity. Antibiotics like penicillin, clindamycin, and vancomycin cause overgrowth of *Candida* species, leading to opportunistic infections [11].

1.4. The mycobiome and metabolic diseases

Obesity and diabetes mellitus type 2, along with other metabolic diseases, are potentiated by dysbiosis in the gut-liver axis. Research has shown a reduced diversity in fungal organisms, an increase in *Candida*, *Nakaseomyces* and *Penicillium* species, and an inverse correlation with *Mucor racemosus* with metrics such as body mass index (BMI) and dyslipidemia. Prostaglandins from *Candida parapsilosis* mediate insulin resistance, hyperglycemia, and lipid deposition, while activation by beta-glycans impairs insulin sensitivity. Other studies revealed a positive association of *Rhodotorula* with high-density lipoprotein (HDL), and negative associations of *Penicillium* with BMI and *Malassezia* with total cholesterol [10,12].

1.5. Effects on the gastrointestinal system

A dysbiosis of *Candida* and *Malassezia* in the gut can promote Inflammatory Bowel Disease (IBD) symptoms, pancreatic adenocarcinoma, and allergic reactions to *Aspergillus*. *Candida* also exhibits mutualistic interactions with *Streptococcus* species in the oral cavity, promoting biofilm formation [13]. *Malassezia* has been implicated in the progression of colorectal cancer.

The bi-directional communication between the gut and the liver has been studied in immune-modulatory responses in multiple liver diseases [14]. Cirrhosis, alcoholic liver disease (ALD), and non-alcoholic liver disease (NALD), all showed decreased microbial diversity as a common feature, with mycobiota dysbiosis. Specifically, candidalysin, produced by *Candida*, induces production of pro-inflammatory cytokines and activation of the Nod Like Receptor Protein 3 (NLRP3) inflammasome, which contribute to severe liver damage in NALD [10]. *Candida albicans* and *C. glabrata* produce triglycerides, and *Saccharomyces* and *Candida* release ethanol to exacerbate NALD.

Studies have shown that the progression of hepatocellular carcinoma could be traced to hepatitis B virus infection and exposure to aflatoxin, produced by *Aspergillus* species [14]. Interactions between *Candida* and *Enterococcus faecalis* have been observed in patients with alcoholic hepatitis.

1.6. Effects on the respiratory system

Early exposure to antibiotics causes microbial dysbiosis that leads to fungal overgrowth, resulting in allergic asthma in children. The presence of *Wallemia mellicola* in the respiratory tract in animal studies underscores the involvement of the mycobiota in severe asthma.

Altenaria-derived serine protease triggers IL-33 activity, leading to asthma pathogenesis [15]. Increased fungal diversity is observed in allergic rhinitis, with higher levels of *Malassezia*. *Aspergillus fumigatus* and *Candida albicans* aggravate symptoms of cystic fibrosis in the lungs. An abundance of *Aspergillus* and *Cryptococcus* is seen in the mycobiota of patients with bronchiectasis. The need to understand the intricacies of the interactions between fungi and bacteria is increased due to new evidence that these biofilms are responsible for antibiotic resistance in the host [16].

1.7. Mycobiota and reproductive health in women

The most common fungal species in the vaginal mycobiota is *C. albicans*, which occupies 70% of the mycobiota. Non-albicans species such as *C. krusei*, *C. parapsilosis*, and *C. tropicalis* are also present. Other commensals include *Saccharomycetales*, *Davidiellaceae*, *Cladosporium*, and *Pichia* [17].

Several studies have examined the interactions of *Candida* species with the *Lactobacillus* bacterial species in the vagina of healthy women [18]. *Lactobacilli* compete with *C. albicans* [19], prevent its adherence to the vaginal wall, and inhibit *Candida* overgrowth by secreting antifungal metabolites. An imbalance in the abundance of beneficial bacteria like *Lactobacilli* could cause dysbiosis. Furthermore, cross-kingdom interactions of fungi with *Streptococcus* and *E. coli* may lead to pre-term birth and sepsis, while *Filobasidium* and *Exophiala* have been associated with intra-uterine adhesion.

Between the first and second trimester of pregnancy, the mycobiota composition shifts towards an increase in *Saccharomyces* enterotype and a decrease in *Aspergillus* enterotype. The genus *Mucor* is associated with gestational diabetes and macrosomia, and pre-pregnancy overweight status has a significant role in the mycobial shift [20].

Thus, women's health is hugely affected by mycobiota dysbiosis, which may suggest targets for probiotic interventions to prevent adverse pregnancy and childbirth outcomes [19].

1.8. Mycobiota and transplant patients

Solid organ transplant and hemopoietic stem cell transplant patients have a high risk for invasive fungal infections, which increase mortality and morbidity and organ rejection [21]. Heart and lung transplant patients are at high risk for *Aspergillus* infections, while liver, small intestine, and pancreas are at high risk for *Candida* infections. Performing careful risk assessments, early detection of fungal infections, customizing therapeutic interventions, and prophylactic antifungal therapy, could increase the chances of a favorable outcome from transplants.

1.9. Mycobiota and cancer

Several fungal species have been implicated in cancer tumorigenesis and progression [22]. *Candida* species contribute to cancer development, progression, and severity through several mechanisms. For example, they synthesize acetaldehyde which contributes to oral cancer in people with chronic alcoholism by promoting inflammation and carcinogenesis through hypermethylation of tumor suppressor genes; form biofilms to protect against immune responses of the host; and exhibit filamentation as a virulence factor that aids cancer progression [23].

Cross-kingdom interactions of fungal organisms *Lichtheimia corymbifera* with specific bacterial taxa *Campylobacter*, *Porphyromonas*, and *Fusobacterium* have been implicated in tongue cancer [24]. Fungal dysbiosis increasing *Malasezzia* interferes with the complement cascade of pancreatic ductal adenocarcinoma, promoting cancer genesis. Thus, modulating this specific interaction of *Malasezzia* could provide therapeutic interventions for pancreatic cancer [25].

Fungal proteins from *Schizosaccharomyces pombi* detected in fecal samples could signal the severity of colorectal cancer. Similarly, in bladder cancer patients, an increase in *Hypocreales*, *Tremellales*, and *Sporidiobiolaies* spp was observed, while *Saccharomyces* was the dominant fungus seen in renal cell carcinoma [26].

Depending on the cancer site, the fungal species, and the metabolic pathway, the host responses and the outcomes were different. Lung cancer had *Blastomyces* as the dominant species, while *Malassezia* species

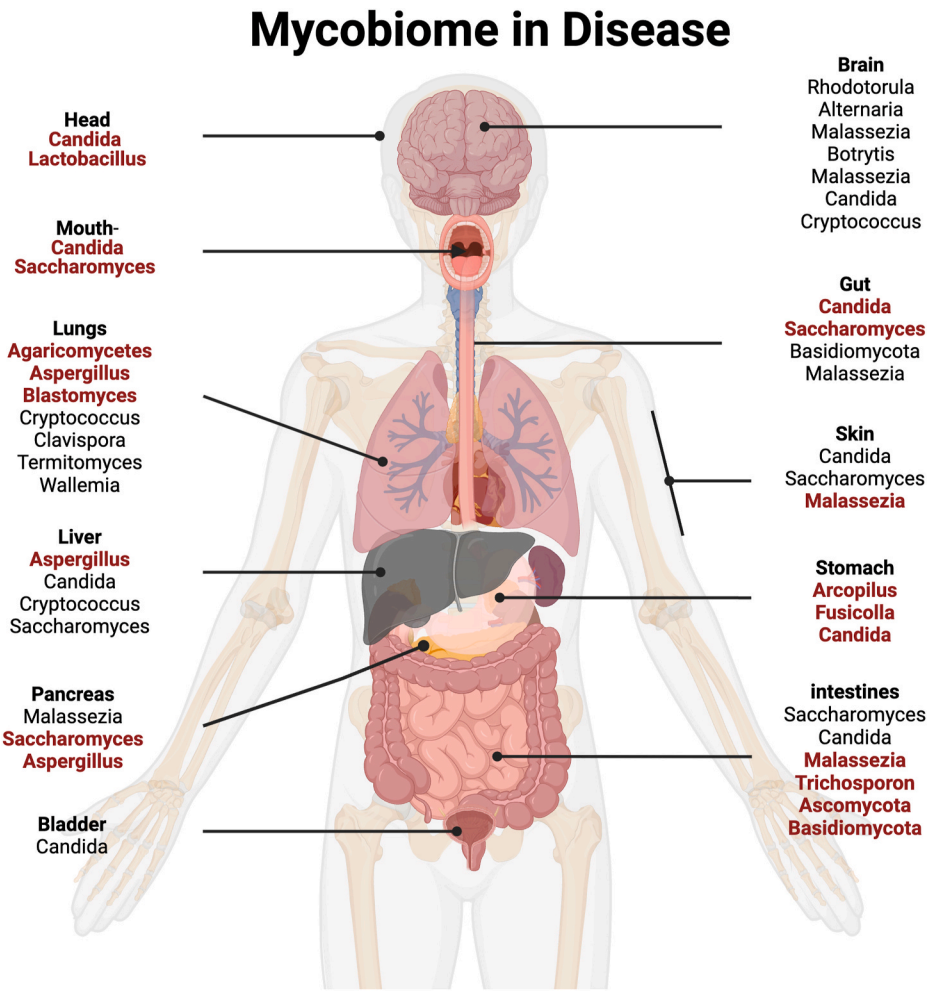

Fig. 1. Diseases affected by the mycobiota. Fungal species have been associated with diseases affecting almost all major organs. Fungi labeled in red play a role in cancer. Figure prepared with BioRender.

were dominant in breast cancer [27]. Gastrointestinal cancers were dominant in either *Candida* or *Saccharomyces*. The detection of *Candida* in gastrointestinal cancer was a predictor of poor survival, while the presence of *Candida* in head and neck and colon cancers indicated an unfavorable prognosis.

Thus, dysbiosis in fungal species can alter cancer therapy outcomes. Understanding these connections would help to shape targeted therapies for cancer relying on the composition of the mycobiota [28].

1.10. Concluding statements

Our understanding of the mycobiome and its effect on human health is far from complete. Researchers continue to delve into the intricate interactions between the mycobiome and the host through dual RNA-sequencing, metabolomics, and proteomics analyses. Integrating multiple omics data streams provides a comprehensive understanding of these complex relationships. Bioinformatics and computational approaches, including specialized analysis pipelines and machine learning algorithms, are invaluable in deciphering the vast quantities of mycobiome data. Furthermore, databases such as FunOMIC now catalog information on the taxonomy and the functions of the human mycobiota [29].

Unveiling the mycobiota's colonization, metabolic interactions, and cross-kingdom dynamics with bacteria, protozoa, and viruses, could revolutionize preventive and therapeutic interventions in precision medicine. Mapping these intricate interactions, combined with interdisciplinary collaborations, could pave the way for novel treatment strategies targeting the mycobiota and the many human diseases that are affected by the mycobiota [Fig. 1].

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