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Review Article

The mycobiome: Role in health and disease, and as a potential probiotic target in gastrointestinal disease

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ABSTRACT

The human gastrointestinal (GI) tract is home to trillions of microorganisms, some beneficial and others potentially harmful. Recent advances in science have allowed us to identify the multitude of organisms inhabiting the GI tract and parse out those that play a role in inflammatory bowel disease (IBD). Unfortunately, most research has focused on studying only the bacteria while ,overlooking a key player, fungus. In order to address this issue, we have focused our efforts on studying the fungal community in the GI tract known as the mycobiome. We found that patients with Crohn's disease (CD) tend to have much higher levels of the fungus Candida tropicalis compared to their healthy family members, as well as two bacteria, *Escherichia coli* and *Serratia marcescens*. Furthermore, we showed that these three organisms worked together to form robust biofilms capable of exacerbating intestinal inflammation. Herein, we discuss the role of the mycobiome in health and disease, and highlight the importance of maintaining balance of the GI microbiota. Additionally, taking into consideration recent next generation sequencing data, we provide insight into potentially new therapeutic approaches in the treatment of IBD through the use of antifungals and/or probiotics aimed at establishing and maintaining a healthy balance of the GI total microbial community including fungi and bacteria.

1. Introduction

For many years, research on the human gastrointestinal (GI) microbiome has focused primarily on resident bacteria and the associated bacterial-host interactions, both beneficial and harmful. Until recently, the role of the human fungal community (mycobiome) in health and disease in the GI tract has been largely untouched, leading to a veritable untapped source of valuable information. In our most recent study [1], we identified a positive correlation between bacteria and fungi, wherein the bacteria *Escherichia coli, Serratia marcescens*, and the fungus *Candida tropicalis* were elevated in the GI tract of patients with Crohn's disease (CD) compared to their non-Crohn's healthy relatives (NCDR). Subsequently, we studied these three organisms in vitro to determine interactions among them. Interestingly, we found that they cooperate in such a way as to form large, robust biofilms capable of activating the host immune response.

These findings suggest a possible role of these pathogenic organisms in the initiation and perpetuation of chronic intestinal inflammation, such as that observed in patients with inflammatory bowel disease (IBD). In a first of its kind study, we have shown that fungi and bacteria cooperate in a strategic way to form pathogenic biofilms capable of initiating an inflammatory response. Not only has this opened up the possibility of new therapeutic approaches in patients with IBD (i.e. antifungals/antibiotics), it has also paved the way for groundbreaking research on probiotic development aimed at disrupting GI biofilm formation, thus ending a vicious cycle of chronic intestinal inflammation.

2. The mycobiome in health

The importance of the mycobiome was highlighted in 2010 in our group's first study describing the oral fungal community using deep-sequencing [2]. The study provided unexpected findings revealing that humans are colonized with numerous fungal species, including the expected (*Candida*), and the unexpected (*Aspergillus, Fusarium, Cryptococcus*). It also showed that, similar to findings in studies investigating the bacteriome, great individual variation in the fungal flora of healthy individuals occurred. Finally, the study showed that over one third of the fungi were un-culturable. These discoveries provide a glimpse of the complex-

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ity in the human microbial flora, which now includes numerous fungal species in addition to the bacteria and viruses, and highlights the need for further investigations into the associations of the mycobiome with health and disease.

Since then, few studies have characterized the mycobiome of the GI tract in health. Studies performed by Dollive et al.[3] and Hoffmann et al.[4] analyzed fecal samples from healthy individuals to identify the most abundant fungal organisms in the GI tract. Dollive et al. described a pipeline for assessing the eukaryotic component of the human microbiome and found that the mycobiome in healthy individuals consisted mainly of *Aspergillus, Cryptococcus, Penicillium, Pneumocystis,* and Saccharomycetaceae yeasts (*Candida* and *Saccharomyces*). The latter study [4] focused on evaluating the effect of diet on the fungal, bacterial, and archaeal components of the GI microbiota. They showed that the fungal phyla Ascomycota and Basidiomycota were mostly inversely correlated, with *Saccharomyces* being the most prevalent genus (present in 89% of the samples), followed by *Candida* (57%) and *Cladosporium* (42%). Interestingly, targeted analysis of the fungi showed a negative association between *Candida* and *Bacteriodes*, with the types of fungal species residing in the gut correlating with the relative proportion of the bacterial taxa and not with the bacterial taxa present per se. Additionally, these authors reported that *Candida* was positively correlated with carbohydrates and negatively with total saturated fatty acids. In contrast, the mould *Aspergillus* was negatively correlated with short chain fatty acids, while no association between *Saccharomyces* and diet was observed.

Studies like these have provided us with insight into the role the mycobiome plays in healthy individuals, and have laid the foundation for future research into how disruption/dysbiosis of the fungal community can lead to disease. It is well understood that maintaining a proper balance of the gut microbiota is essential for overall GI health [5]. We have drawn focus on the mycobiome in particular in an effort to increase more studies like the ones discussed herein to further investigate the role of the mycobiome in GI health.

3. The mycobiome in IBD

The two most common forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). CD is a relapsing, transmural inflammatory disease of the GI mucosa that can affect the entire GI tract from mouth to anus, whereas UC is a relapsing non-transmural inflammatory disease that is restricted to the colon [6]. The pathogenesis of CD is thought to arise from an inappropriate immune response to the intestinal microbiota, resulting in inflammation of the GI mucosa. Countless studies have shown how even slight disruptions in that microbial balance can lead to aberrant immune responses, invoking inflammation, and a long list of other deleterious side effects.

Association between fungi and the GI tract has been documented since the 18th century, with a special focus on candidiasis [7]. One of the first reports of this association was the study by Rosenstein, who described oral candidiasis that extended to the stomach and intestines. Additionally, the first reported case of GI candidiasis in an infant was described during this period. Subsequent publications in the 19th century documented *Candida* infection of the stomach, colon and ileum [8,9].

3.1. Importance of selecting the appropriate control cohort

Until recently, the only study investigating the mycobiome and IBD was by Illiev et al.[10]. Using a dextran sodium sulfate (DSS)-induced colitis mouse model, these authors showed that: 1) the gut is colonized with 10 fungal species dominated by *C. tropicalis* (65%), and 2) fungi and NOT bacteria are responsible for aggravating the severity and inflammation of IBD, rather than a cause of these symptoms, which clearly demonstrates the contribution of the mycobiome to colitis.

Table 1
Abundance of identified fungal phyla, class and order in CD (disease) and NCDR (healthy relatives) groups.

Taxon name		CD		NCDR		*P*
		Mean	SD	Mean	SD	
Phylum	Ascomycota	78.99%	17.32%	74.45%	19.10%	0.122
	Basidiomycota	0.37%	0.69%	3.44%	15.55%	0.353
	Glomeromycota	0.06%	0.10%	0.85%	2.24%	0.176
	Zygomycota	0.39%	0.95%	0.31%	0.68%	0.172
Class	Archaeorhizomycetes	0.00%	0.00%	0.88%	4.66%	0.398
	Dothideomycetes	0.24%	0.51%	0.21%	0.45%	0.334
	Eurotiomycetes	5.68%	9.97%	3.92%	7.67%	0.326
	Leotiomycetes	0.15%	0.41%	0.09%	0.19%	0.418
	Saccharomycetes	54.23%	15.24%	50.12%	17.39%	0.477
	Sordariomycetes	0.55%	1.44%	2.79%	13.94%	0.520
	Agaricomycetes	0.77%	1.21%	0.57%	1.67%	0.866
	Tremellomycetes	0.21%	0.48%	0.06%	0.16%	0.744
	Wallemiomycetes	0.00%	0.01%	0.08%	0.40%	0.765
	Glomeromycetes	0.06%	0.10%	0.85%	2.24%	0.183
Order	Archaeorhizomycetales	0.00%	0.00%	0.56%	2.96%	0.398
	Capnodiales	0.09%	0.23%	0.14%	0.30%	0.189
	Pleosporales	0.14%	0.42%	0.07%	0.19%	0.789
	Chaetothyriales	0.02%	0.04%	0.17%	0.89%	0.183
	Eurotiales	5.65%	9.99%	3.71%	7.53%	0.490
	Verrucariales	0.00%	0.00%	0.00%	0.00%	1.000
	Saccharomycetales	53.71%	14.86%	49.12%	16.59%	0.439
	Hypocreales	0.47%	1.39%	0.10%	0.10%	0.915
	Agaricales	0.04%	0.09%	1.30%	5.28%	0.060
	Boletales	0.11%	0.18%	0.44%	1.44%	0.277
	Cantharellales	0.24%	0.60%	0.03%	0.05%	0.700
	Corticiales	0.05%	0.20%	0.04%	0.21%	0.765
	Hymenochaetales	0.10%	0.43%	0.00%	0.00%	0.237
	Polyporales	0.07%	0.21%	0.02%	0.04%	0.759
	Russulales	0.16%	0.70%	0.03%	0.10%	0.212
	Thelephorales	0.00%	0.00%	0.00%	0.00%	0.227
	Malasseziales	0.00%	0.00%	0.00%	0.01%	0.398
	Tremellales	0.20%	0.48%	0.03%	0.11%	0.209
	Wallemiales	0.00%	0.01%	0.08%	0.40%	0.765
	Archaeosporales	0.00%	0.01%	0.79%	2.23%	0.161
	Glomerales	0.02%	0.03%	0.04%	0.08%	0.140
	Mucorales	0.38%	0.94%	0.29%	0.63%	0.186

We have extended the work from animal models to patients with CD as described in our most recent study [11]. Since CD is a disease mediated by a multitude of factors including host genotype, dysregulated immune response, and the intestinal microbiota, and since members of a family share genetics, environment, diet and bacterial microbiota and are more similar to each other than they are to unrelated individuals [11], we decided to characterize the mycobiome and bacteriome in CD patients in multiplex families compared to their unaffected first-degree relatives (NCDR). Table 1 summarizes the fungal profile in healthy and CD patients. We used unrelated healthy individuals as a control to limit the influence of both genetic background and diet. Our analysis confirmed and extended the findings of Schloss et al.[11], demonstrating that the diversity of gut microbial communities of both CD patients and their first-degree healthy relatives were distinct from the unrelated healthy individuals. These findings emphasize the importance of selecting the appropriate control so as to reduce confounding factors.

3.2. *Elevation of bacteria and fungi in Crohn's disease*

Comparison of the bacterial and fungal communities between CD patients and their NCDR demonstrated significant differences in the taxa of these communities between the two groups. For example, in agreement with previous studies [12–16], bacteria belonging

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