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Review article: fungal alterations in inflammatory bowel diseases

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Summary

Background: Emerging data suggest that alterations in gut fungi may be associated with the pathogenesis of inflammatory bowel disease (IBD). In healthy individuals, gut commensal fungi act synergistically with other members of the microbiota to maintain homeostasis but their role in IBD is less clear.

Aim: To review the role of gut fungi and their trans-kingdom interactions with bacteria in IBD

Methods: A literature search was conducted on Ovid and Pubmed to select relevant animal and human studies that have reported fungi and IBD.

Results: There is an increased total fungal load particularly of *Candida* and *Malassezia* species in the faeces and mucosa of Crohn's disease patients, and a lower fungal diversity in the faeces of ulcerative colitis patients. Caspase recruitment domain-containing protein (CARD)-9 polymorphism in Crohn's disease patients favours *Malassezia* colonisation that worsens gut inflammation. Diet high in carbohydrates increased the total abundance of *Candida* species, whereas protein-rich diet had the opposite effect. Anti-fungal therapies are mostly used to treat *Candida albicans or Histoplasma capsulatum* infections in IBD, whereas pilot studies of supplementing fungal probiotics *Saccharomycopsis fibuligera*, *Saccharomyces boulardii* and *Saccharomyces cerevisiae CNCM I-3856* strain showed therapeutic effects in IBD.

Conclusions: Gut fungi are altered in patients with Crohn's disease and ulcerative colitis. Modulation of the fungal microbiota can be considered as a therapeutic approach for IBD. Future research should focus on understanding how the fungal microbiota interacts with other components of the gut microbiota in association with the pathogenesis and development of IBD.

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1 | BACKGROUND

The incidence of Crohn's disease (CD) and ulcerative colitis (UC), the two subtypes of IBD, is rising rapidly worldwide particularly in newly industrialised countries. However, the cause of this remains unclear.[1,2] Aetiological studies on IBD have elucidated the role of genetics, host immune response, gut microbiota and environmental triggers in disease pathogenesis.[3] Genetic loci including Nucleotide-binding oligomerisation domain-containing protein 2 (*NOD2*), interleukin 10 (*IL-10*) and Caspase recruitment domain-containing protein 9 (*CARD9*) have been associated with IBD,[4-10] but their effects on populations of different ethnicities and geography are heterogeneous.[1,11,12] Alterations in the gut microbiota and changes in environmental factors most likely account for the rapid emergence of IBD globally.[13] To date, tremendous efforts have been focused on delineating the role the of bacterial microbiome in IBD,[14] while studies of other components of the gut microbiota, including changes in fungi or "mycobiota" are scarce.[15] Although fungi only constitute approximately 0.1% of the total microorganisms in the gut,[16-18] they have been thought to also play a role in IBD pathogenesis.[19-23] In addition to intestinal inflammation, external factors including diet, antibiotics and immunosuppressive therapy can influence the structure or composition of mycobiota in patients with IBD. In this review, we discuss alterations in the mycobiota, also known as "fungal dysbiosis" in IBD, trans-kingdom interaction between fungi and other members of the gut microbiota and their potential role in the pathogenesis of IBD. We highlight the role of fungi and their functions in animal and human IBD and illustrated how environmental factors impact on the gut mycobiota.

2 | ADVANCES IN MYCOBIOTA PROFILING IN HUMANS

The advent of more affordable, high-throughput sequencing technologies has improved our understanding of the fungal composition in humans. Five commercial extraction kits are frequently used for DNA extraction: QIAamp Fast DNA Stool Mini Kit (Q), Q and Bead beating, Q and Lyticase lysis buffer, FastDNA® SPIN Kit and Repeat bead beating plus column (RBBC).[24] Q gives a relatively low DNA yield but higher purity, whereas FastDNA® SPIN Kit provides a higher DNA yield, but lower purity because the product is mixed with high levels of proteins.[24] To optimise extraction quality, bead beating combined with lyticase digestion is recommended to enhance DNA release from fungal cell wall lysis,[24,25] and column-based DNA purification techniques can enhance the end-product yield and purity.[24,25]

After DNA extraction from faecal samples, the sequencing platform is another factor that affects mycobiota evaluation. Direct sequencing of fungal DNA has been the main method for characterising the mycobiota. Common sequencing targets include the 18S small-subunit ribosomal DNA (rDNA) and 28S large-subunit rDNA. Traditional metagenomic sequencing lacks resolution in evaluating the mycobiota composition in the human gut[26] due to the dominance of bacterial community and that gut fungi only constitute approximately 0.1% of the gut microbial communities.[16-18,27] Deeper metagenomic sequencing and the extension of fungal databases can enhance sequencing output and fungal species identification.[28] Due to current limitations of metagenomic sequencing, a more objective metabar coding technique known as ITS (internal transcribed spacer) sequencing is routinely implemented to specifically target the mycobiota.[29,30] However, mis-attribution of sequences and classification of sexual and asexual forms of the same fungus are current challenges in characterising fungal populations using next-generation DNA sequencing technology. Unlike bacterial 16S rDNA sequences whereby large databases have been developed, the sequences for fungi available in the NCBI GenBank database are far from complete and estimates suggest that only less than 1% of fungal species are currently represented.[31]

The mammalian colon harbours the highest concentration of fungal organisms.[22] The most abundant fungal genera in healthy individuals are *Candida*, *Saccharomyces* and *Cladosporium*.[22,32] Intestinal or faecal mycobiota appears to be less stable and more susceptible to episodic fluctuations than bacterial microbiota.[32] More recently, fungi composition in healthy intestinal mucosa were elucidated and the phyla *Ascomycota* and *Basidiomycota* were shown to be the most abundant taxa in the mucosal samples of healthy individuals.[33] The classes *Saccharomycetes* and *Tremellomycetes* are dominant in the phyla *Ascomycota* and *Basidiomycota* respectively. Further classification at a lower taxonomic level showed that these two classes could be subdivided into the *Candida*, *Debaryomyces*, *Saccharomyces*, *Malassezia*, *Sporobolomyces*, *Trichosporon*, *Wallemia* genera, along with a smaller proportion of unidentified *Filobasidiaceae* and *Xylariales*.[33]

3 | FUNGAL ALTERATIONS IN FAECAL AND MUCOSA SAMPLES IN IBD

Several fungal taxa appeared to be consistently altered during chronic intestinal inflammation. The first human study investigating gut fungal composition of IBD in 2008 used denaturing gradient gel electrophoresis (DGGE)[34] and found an increased diversity of fungi in faecal samples of CD patients. However, the study could not link individual fungal species to IBD due to the lack of sequencing resolution.[34] In 2015, high-throughput 16S ribosomal RNA and Internal transcribed spacer 2 (ITS2) sequencing were performed to evaluate the fungal composition in paediatric IBD faecal samples[27,35] and these studies consistently showed an elevated abundance of *Candida* species.[27,35] More recently, fungal composition of the faecal microbiota of 235 patients with IBD and 38 healthy subjects was determined using ITS2 sequencing[30] to show an increase in *Basidiomycota*-to-*Ascomycota* ratio. One of the most striking features was the increased abundance of Basidiomycota in IBD and particularly during disease flare, which was balanced by an equivalent decrease in Ascomycota.[30] Basidiomycota and Ascomycota

TABLE 1 Gut fungi that have been reported to be associated with IBD

Fungi	In mice	In humans	References
Candida albicans	Not present in mice	Higher prevalence in faeces of CD patients and their healthy relatives, might correlate with ASCA level	43,107
Malassezia restricta	Supplementation exacerbates colitis	Exacerbates inflammation in mucosa of CD patients who have CARD9^{S12N} polymorphism	38
Candida tropicalis	Increased in faeces; Exacerbates colitis and airway allergy	Higher prevalence in faeces of CD patients	58
Candida glabrata	Increased in faeces; Exacerbates colitis and airway allergy	Higher prevalence in faeces of CD patients	31,33
Aspergillus amstelodami	Stimulated after 1-3 wk fluconazole administration, exacerbates colitis and airway allergy	Unknown	108
Epicoccum nigrum	Stimulated after 1-3 wk fluconazole administration, exacerbates colitis and airway allergy	Unknown	108
Wallemia sebi	Stimulated after 1-3 wk fluconazole administration, exacerbates colitis and airway allergy	Unknown	108
Saccharomyces cerevisiae	Decreased in faeces of mice with colitis; Daily supplementation increases purine metabolism and exacerbate colitis	Decreased in faeces and mucosa of IBD and during IBD flare, increased in faeces of healthy individuals and IBD in remission	30,99
Dioszegia genus	Unknown	Increased in the inflamed mucosa of IBD	22,33
Leptosphaeria genus	Unknown	Decreased in the non-inflamed mucosa of IBD	22,33
Trichosporon genus	Increased in mice with colitis	Decreased in non-inflamed mucosa of IBD	22,33
Filobasidium uniguttulatum	Unknown	Increased in non-inflamed mucosa of IBD	22,33
Xylariales genus	Unknown	Increased in inflamed mucosa of CD	22,33

abundances exhibited a strong negative correlation with each other.[30] Although *Ascomycota* was skewed in this ratio, the relative abundance of *C albicans* under the *Ascomycota* phyla was increased as its absolute quantity was stably unchanged as shown by quantitative PCR reaction.[30] This result suggested that despite a substantial proportional reduction of *Ascomycota*, *C albicans* exist in this phylum and colonise normally during inflammation.[30] Table 1 describes the fungal species that have been reported to be associated with IBD.

Interestingly, there was a negative correlation between the abundance of *S cerevisiae* and *C albicans* in faecal samples of IBD subjects, suggesting a competition between the two species in the gut.[30] *S cerevisiae* has been shown to compete with the colonisation and adhesion of *C albicans* and by suppressing the expression of secreted aspartyl proteases (*SAPs*) - *2* and *6* in *C albicans*, it prevents *C albicans* from transforming into its invasive hyphal form.[36,37]

The abundance change of gut fungi is not only restricted to the faecal microbiome but also occurs in the diseased-mucosa.[38] A mucosa-associated fungus called *Malassezia restricta* (*M restricta*) that normally presents in skin and gut mucosa was found in CD mucosa.[38] *M restricta* has been found to worsen gut inflammation particularly in CD patients who have Caspase recruitment domain-containing protein (CARD)-9^{S12N} polymorphism, suggesting that CARD9 signalling is critical for *M restricta* sensing.[38] This hypothesis is supported by exacerbated colitis progression when *M restricta* was colonised in wild type and germ-free mice.[38] Various C-type lectin receptors, Mincle, CARD9 and Dectin-2 have been found to be responsible for *M restricta* recognition,[38-40] but the function of CARD9^{S12N} remains unknown, and it was found to be associated with several autoimmune diseases, such as IBD and asthma.[38,40] CARD9^{S12N} can facilitate interleukin 5 (IL-5) secretion in alveolar macrophages for a type II immune response but its central role in CD pathogenesis is not understood.[38,40]

4 | IMPACT OF FUNGAL ALTERATIONS ON IBD DIAGNOSIS AND DISEASE ACTIVITY

4.1 | Disease diagnosis

Mycobial profiling can potentially help to identify and differentiate fungal compositions between IBD patients and healthy controls. Upon targeting the responsible fungi from the sequencing profile, mycobiota-based IBD diagnosis may become possible. Currently, the antibody—Anti-*Saccharomyces cerevisiae* antibody (ASCA) has been used to target *S cerevisiae* cell wall antigens, mannose alpha 1,3 mannose, for the diagnosis of CD.[41] Nevertheless, *C albicans* also express common β-glucan epitopes similar to that of *S cerevisiae*, suggesting that ASCA as a diagnostic tool may not be specific enough to detect individual fungi.[27,42,43] Various sources of microbes and receptors that express these ubiquitous epitopes might also affect ASCA affinity binding, including mycobacteria *M paratuberculosis* and the fimH

receptor on GP2 on M cells, all of which likely contain the mannose alpha 1,3 mannose epitope for ASCA.[44-47] When serological markers are considered individually, ASCA has the best combined sensitivity and specificity for CD; and pANCA for UC. It has been demonstrated that these two antibodies in combination are more accurate in differentiating CD from UC than when used in isolation.[48]The specificity of ASCA is particularly high in CD, around 41%-76%,[43,49,50] and it is often used to differentiate between CD and UC when the diagnosis is unclear. ASCA is also found more commonly in CD patients with a family history of IBD,[43,49,50] single nucleotide polymorphisms in CARD9 or dectin-1 could be applied to diagnose susceptibility loci in potential subjects.[22,51,52] *C albicans* is more likely to colonise CD patients (44%) and their first-degree healthy relatives (FDR) (38%) compared to healthy individuals (22%).[43] Although CD patients and their FDR have a higher burden of *C albicans* colonisation, it is reflected by a higher prevalence of ASCA but not a significant elevation of total antibody level. It is unclear if the increased *C albicans* colonisation directly contributes to disease activity.[43] *S cerevisiae* detection in faecal samples based on quantitative PCR was found to be decreased in IBD and during IBD flare but increased in faecal samples of healthy control and IBD subjects in remission.[30] Compared to *C albicans*, *S cerevisiae* has a more substantial role in IBD activity.[30]

4.2 | Disease activity or severity

Overall, CD patients experience a higher fungal burden during the inflammatory process, while UC patients have a decreased diversity of gut fungi.[30,53-55] In CD, altered ileal physiology in the terminal ileum impairs the inhibitory effect of antimicrobial peptides on bacteria and bile acid reabsorption.[30,53-55] This altered ileal physiology in CD, which is not present in UC, may facilitate fungal colonisation in the terminal ileum.[30,53-55] This may explain why an increased load of *Candida* species is a distinctive feature in CD; this increase also correlated with disease activity and severity.

Compared to healthy individuals, the total fungal load was more prominent in the mucosa of CD patients with a 40-fold increase during disease flare.[31] The diversity of genus *Dioszegia* and *Candida* was also increased, particularly the expansion of *Candida glabrata* in the inflamed mucosa; and a concurrent increase in *S cerevisiae* abundance in the non-inflamed mucosa. The genera *Leptosphaeria* and *Trichosporon* were decreased[31] (Table 1).[33,43,56,57] In addition, *Filobasidium uniguttulatum* were elevated in the non-inflamed mucosa, whereas *Xylariales* were increased in the inflamed mucosa of CD.[33] Increased relative abundance of *S cerevisiae* and *C glabrata* was observed,[33] and a positive correlation between *Candida tropicalis* and familial CD was also reported.[58] Similar alterations of these two commensal fungi have been reported in patients with immunocompromised gastrointestinal (GI) tract or Irritable bowel syndrome.[59-61]

What remains unknown is whether changes in gut fungi diversity are a cause or a consequence in IBD. Such insights require mechanistic studies in animals, and future work should investigate factors that affect gut fungal colonisation in the gut.

5 | CLINICAL STUDIES OF ANTI-FUNGAL THERAPIES AND FUNGAL PROBIOTICS IN IBD

With documented alterations in gut mycobiota on IBD activity based on data on mycobial profiling, detection and ASCA and quantitative PCR analysis,[30,43] modulation of the fungal community may offer therapeutic approaches to IBD. Fluconazole, an anti-fungal drug, is not a common treatment for IBD, and it is mostly used to eradicate fungal infection, candidiasis or candidaemia in immunocompromised IBD patients.[62,63] IBD patients with single nucleotide polymorphisms and mutations in the major genetic loci have altered function of pathogen-associated molecular patterns (PAMPs) and cytokine production and are more susceptible to fungal infections. Table 2 shows the genetic mutations and single nucleotide polymorphisms associated with increased susceptibility to fungi infections in IBD. In a study of 89 patients with UC, fluconazole was used to treat a group of 20 patients who have been diagnosed with significant fungal colonisation. The fluconazole-treated group showed a significant reduction in the UC activity index compared with other groups treated with mesalazine (mesalamine), azathioprine or probiotic lacidofil.[64] Three pilot studies showed that amphotericin B and itraconazole were promising to treat *Histoplasma capsulatum* infection in IBD.[65-67] Two studies showed that amphotericin B and itraconazole significantly reduced or completely eradicated *H capsulatum* in CD; [65,66] one study showed that itraconazole relieved symptoms and attained clinical and endoscopic remission among 67% of IBD patients.[67]

In addition to fungal eradication, replenishing fungal probiotics is another approach that modulates the gut mycobiota. A pilot study of 24 UC patients who had mild to moderate flares and were receiving mesalazine were additionally administrated 250mg of *S boulardii* three times per day for 4 weeks.[68] Overall, 17 of 24 patients achieved clinical and endoscopic remission at 4 weeks.[69] Two studies reported supplementing *S boulardii* to CD patients. In one study, 32 patients with CD in clinical remission were randomly assigned to either mesalazine alone or mesalazine plus a preparation of *Saccharomyces boulardii* 1 g daily for 6 months Clinical relapse based on CDAI was significantly lower in the mesalazine plus probiotic group compared with mesalazine only group (6% vs 38%).[70]A separate study showed that *S boulardii* significantly reduced stool frequency in 20 CD patients compared to baseline.[71] Table 3 shows the clinical studies of anti-fungal therapies and fungal probiotics in IBD.

Mechanistic studies showed that *S boulardii* reduced local inflammation by restricting movements of dendritic cells to inflammatory sites.[22,72,73] *S boulardii* also induced Interleukin 8 (IL-8), Interferon gamma (IFN-γ) and Transforming growth factor beta (TGF-β) secretion and helper T cells deposition in lymph nodes, thereby reducing the total number of T cells.[22,72,73] Another fungal probiotic—*Saccharomycopsis fibuligera* has emerged as a *Saccharomyces* strain that showed therapeutic effect in mice but this newly patented probiotic has not been applied to human studies.[22]

TABLE 2 Genetic mutations and SNPs associated with increase susceptibility to fungal infections in IBD

Gene	Increase *candida* infection risk	Affected in IBD	Mutation/SNP (rs-number)	Functions/systemic response	References
CARD9	Yes	Yes (susceptible to *Candida dubliniensis* infection)	G72S, R373P, Q295X	T_h17 level ↓	109,110
Dectin-1	Yes	Yes	Y238X (rs16910526)	IL-1β and T_h17 responses↓	111,112
IL-10	Yes	In CD particularly	-1082A/G (rs1800896)	IL-10 production ↓, persisting *candida* infection	113,114
IL-4	Yes	Unclear (but much susceptible to *C difficile* infection in IBD)	-589C/T (rs2243250)	Unknown	115,116
PTPN22	Yes	In CD particularly Inconclusive in UC	R620W (rs2476601)	Gain-of-function suppression of T-cell activation	117-119
TLR1	Yes	Yes	R80T (rs5743611) S248N (rs4833095) I602S (rs5743618)	Production of IL-1β, IL-6 and IL-8↓ after TLR1-TLR2 signalling	30,120,121
TLR3	Yes	Yes (also linked with *S cerevisiae* susceptibility)	L412F (rs3775291)	IFN-γ level ↓	30,122,123
TLR4	Yes	Yes (also susceptible to Mycobacterium avium subspecies paratuberculosis (MAP) – positive CD)	D299G (rs4986790) Y399I (rs4986791)	IL-10 level ↑	124-126
CX3CR1	Yes	In CD particularly	T280M (rs3732378)	IgG level ↓	127
CARD9	Unknown but responsive to *Aspergillus fumigatus*	In CD particularly	$CARD9^{S12N}$	NF-κβ subunit (RelB) level ↑ IL-5 production ↑	38,40

6 | INFLUENCE OF DIET ON GUT FUNGI COMPOSITION AND ABUNDANCE

Increasing evidence from animal, clinical, and epidemiological studies suggests that diet high in carbohydrates or animal fat, and low in fibre, is associated with an increased risk of IBD in genetically susceptible individuals. Modifying diets may reduce the risk of disease development or flare.[74] Diet has been shown to affect the fungal microbiota. In healthy individuals, a plant-based diet was associated with an increase in gut colonisation by *Candida* species, whereas the intake of an animal-based diet facilitated the expansion of *Penicillium* species.[75] Carbohydrates are a robust energy source for fungi and serve as a sugar component for cell wall remodelling and act as a tryptophan precursor when starved.[29,76-78] Studies showed that *Candida* species degraded complex carbohydrates and starch via fermentation to provide simpler sugars as an energy source to different types of microbes.[79,80]

Amino acids and proteins can impact the abundance of *C albicans* differently in in vivo and in vitro models.[29,81] In an in vivo model, Hoffmann *et al* reported a negative association between the abundance of *C albicans* and amino acid uptake.[29] In contrast, an in vitro model by Miramón and Lorenz showed that *C albicans* were positively associated with amino acid consumption because the fungus converts amino acids into carbohydrates. The newly formed carbohydrates were used to neutralise the acidic environment of the phagolysosome, thereby subverting macrophages' cytotoxicity.[81] These two distinct results suggest that amino acids serve as a nutrient source for *C albicans;* fungi with higher possession of amino acids are more likely to survive in the gut, provided these fungi can convert amino acids into carbohydrates and favour a relatively alkaline environment.[29,81] Competition for amino acid has been reported for *C albicans* and *Lactobacillus*, a Gram-positive bacterial population that was found to be suppressed in patients with IBD.[76,82,83] High consumption of dietary protein by the host led to the breakdown of tryptophan forming indoles via indoleamine 2,3-dioxygenase 1 (IDO1) secreted by *Lactobacillus* in the gut.[76,83] Aryl hydrocarbon receptor from the host was activated by the newly formed indoles. The activated Aryl hydrocarbon receptor further up-regulated Interleukin 22 (IL-22) and T-helper cell 17 to inhibit *C albicans* colonisation (Figure 1).[76,83]

TABLE 3 Clinical studies of anti-fungal therapies and fungal probiotics in IBD

Drugs	Disease subtypes	Number of patients	Target	Infection or colonisation	Clinical outcome	References
Fluconazole	UC	20	*Candida albicans*	Colonisation	UC activity index reduced	[64]
Amphotericin B	CD	4	*Histoplasma capsulatum*	Infection	Recovered from histoplasmosis	[65]
Itraconazole or Amphotericin B	Paediatric CD	5	*H capsulatum*	Infection	1 lost to follow-up, 4 showed reduced or negative histoplasma antigens	[66]
Itraconazole	CD and UC	5 CD, 1 UC	*H capsulatum*	Infection	4 of 6 attained clinical and endoscopic remission, and were able to withdraw immunosuppressants	[67]

Fungal probiotics	In mice	In human	References
Saccharomycopsis fibuligera	Ameliorates colitis in mouse models	Unknown	[22]
Saccharomyces boulardii	Ameliorates colitis and lowers *C albicans* colonisation	UC: 17 of 24 patients achieved clinical and endoscopic remission at 4 wk with *S boulardii* CD: Clinical relapse rate lower in 32 patients who had *S boulardii* supplementation CD: *S boulardii* significantly reduced stool frequency in 20 patients compared to their baseline	[22,68,70-73,128]
Saccharomyces cerevisiae CNCM I-3856 strain	Opposes adherent / invasive *Escherichia coli* (AIEC) colonisation in mice of colitis	Unknown	[106]

Dietary fatty acids have been shown to affect the abundance of *C albicans*. For instance, high consumption of saturated fat increases taurine's conjugation to bile acid in the live, which forms hydrogen sulphide.[84,85] Hydrogen sulphide is the major source of organic sulphur that promotes *Lactobacillus* growth in the gut,[84-86] and exerts an inhibitory effect on *C albicans* leading to a reduced abundance (Figure 1).[84-86] Besides, short chain fatty acids can directly inhibit *C albicans*.[87] Conjugated linoleic acid was also reported to block *C albicans* transformation from yeast to the hypha form.[87] Conjugated linoleic acid impedes GTP-binding protein Ras1p's anchorage onto the intracellular membrane, and also suppresses mRNA and protein levels of GTPase *RAS1*, a key component of *C albicans'* cell cycle for hyphal transformation.[87]

7 | POLYMORPHIC PLASTICITY OF *C ALBICANS* IN HEALTH AND DISEASED ANIMAL MODELS

In the mammalian gut, environmental and nutritional stresses cause *C albicans* to undergo morphological transformation.[88] The healthy intestinal environment triggers a phenotypic transformation of *C albicans* by up-regulating the transcription factor *White-opaque regulator 1* (*WOR1*), which is exclusive to *Candida* species.[88] The adapted form of *C albicans* is termed Gastrointestinal-indUced Transition (GUT)[88] (Figure 2). Compared to the original yeast form, the GUT form is able to up-regulate genes responsible for fatty acid and *N*-acetylglucosamine metabolisms whilst down-regulating genes for iron uptake and glycolysis.[88] The polymorphic plasticity allows *C albicans* to thrive in blood and GI niches, and also allows *C albicans* to possess an evolvable iron regulatory network to switch between commensalism and virulence depending on its niche.[89] The evolutionary insertion of this transcriptional activator Sef1 promotes iron uptake and virulence in the bloodstream, whereas the indigenous Sfu1 transcriptional repressor allows *C albicans* to repress iron uptake and avoid iron toxicity in GI niches.[89] It can be inferred that when the GUT form is present, fatty acids are utilised, glycolytic rate is decreased to reduce energy expenditure, and iron toxicity is avoided.[88-90] To date, only *C albicans* has been reported as the only fungus to possess such characteristics in the mammalian gut, and the presence of GUT form may account for the dominance of *C albicans* in the gut amongst other intestinal

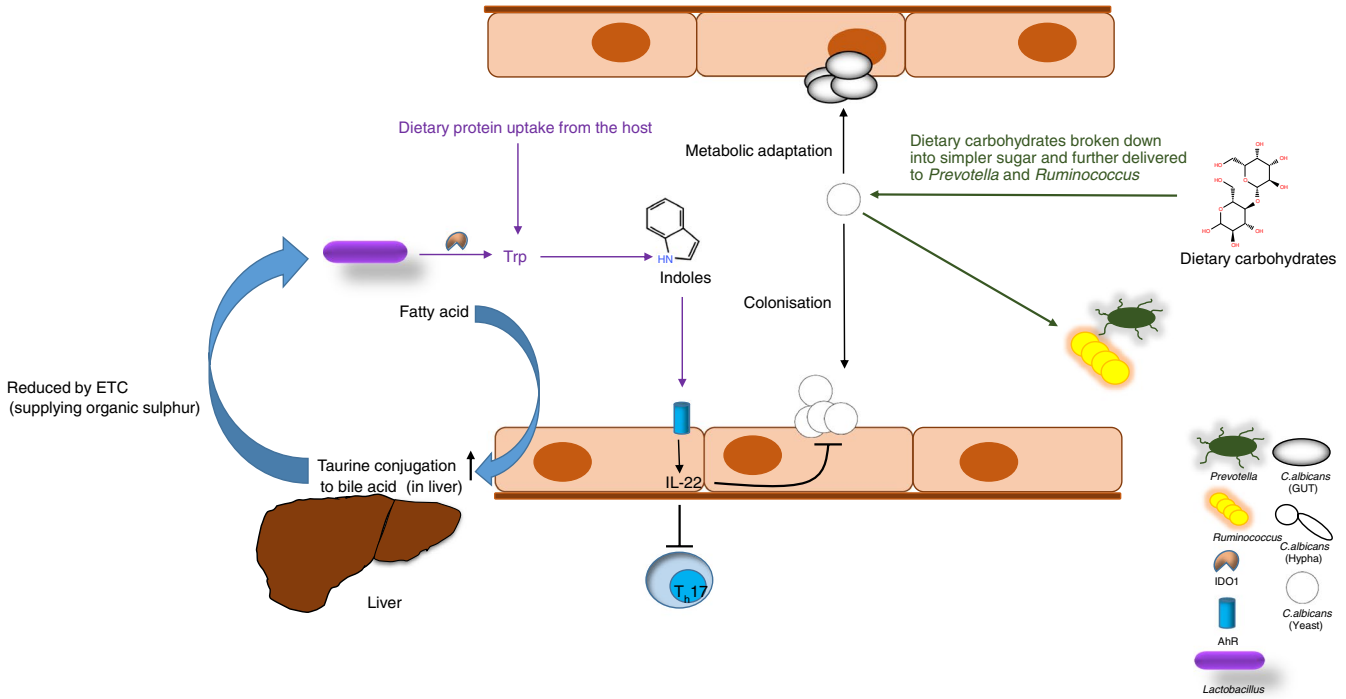

FIGURE 1 Association between host diet consumption and microbial metabolism. Complex sugar, carbohydrate and starch consumption provides energy source for the gut microbiota. *Candida albicans* degrades complex carbohydrates via fermentation, which provides simpler sugar as an energy source for inter-species *Prevotella* and *Ruminococcus*. Alternatively, carbohydrate is utilised by *C albicans* for cell wall remodelling and maintenance. On the other hand, fatty acid consumed by the host provides high saturated fat to the bacterial genus *Lactobacillus*. Fatty acids also increase taurine-conjugation to bile acid in the host liver, which is further reduced by accepting electrons from electron transport chain (ETC) to form an end-product hydrogen sulphide, supplying organic sulphur to the bacterial community. Other dietary components such as proteins are broken down to tryptophan, and they are taken by *Lactobacillus*. *Lactobacillus* breaks down tryptophan into indoles via indoleamine 2,3-dioxygenase 1 (IDO1). Indoles bind and activate the aryl hydrocarbon receptor (AhR) that up-regulates interleukin 22 (IL-22) and down-regulates T helper 17 cells (Th17) cells in opposing *C albicans* colonisation

fungi.[88-90] In a healthy mammalian gut, *C albicans* is exclusively in the GUT form[88,91,92] (Figure 2). While in an immunocompromised host, *C albicans* is found to be in its hyphal form which preferentially colonises and invades the host mucosa by secretion of aspartyl proteases.[91-93] Expression of enhanced filamentous growth protein 1 (*EFG1*), a transcription factor responsible for yeast-to-hyphal transition, was found to be significantly elevated in the immunocompromised gastrointestinal (GI) tract, indicating that the condition of host immunity is linked to the morphological state of *C albicans*.[60,91,92] A recent study also showed that the transformation of *C albicans* from yeast to hyphae overexpresses candidalysin, a fungal peptide toxin associated with mucosal damage.[94] There remains a lack of data to describe the morphological plasticity of *C albicans* or other fungi in IBD.

8 | HOST RESPONSE TO FUNGAL ALTERATIONS IN IBD ANIMAL MODELS

In a homeostatic state, fungal-host communications work harmoniously with other trans-kingdom microbes in the gut.[17] Dysbiosis of the mycobiota disrupts this communication and results in pro-inflammatory responses.[19,30,95] Several murine models of colitis have been used to interrogate the interplay between gut fungi and the host. Iliev *et al* showed that polymorphism of the gene *CLEC7A* encoding for Dectin-1 was significantly associated with UC severity in mice with predisposed susceptibility to intestinal colitis due to the disruption of fungal sensing.[22] The Dectin-1 signalling cascade was initiated by β-glucan recognition in fungal cell walls.[22,96,97] Ligand binding further signals Spleen tyrosine kinase (SYK) phosphorylation and CARD9 activation.[22,96,97] Activated CARD9 protein then activates Apoptosis-Associated Speck-Like Protein Containing CARD (ASC) adaptor molecules and caspase 1 which enzymatically cleaved pro-interleukin 18 (IL-18) into mature IL-18 in promoting epithelial cell proliferation and restitution.[22,96,97] Additionally, CARD9 activation was also coupled with binding of ubiquitin ligase—Tripartite Motif Containing 62 (TRIM62).[98] Mice with Trim62$^{-/-}$ that were challenged with Dextran Sodium Sulfate (DSS) -induced colitis had increased susceptibility to *C albicans* infection, suggesting that the absence of TRIM62 binding likely suppressed CARD9 ubiquitination, which consequently silenced the downstream CARD9 signalling.[98] In Illev's model, ITS1 sequencing data showed a similar pattern with Sokol et al,[22,30] whereby gut inflammation led to

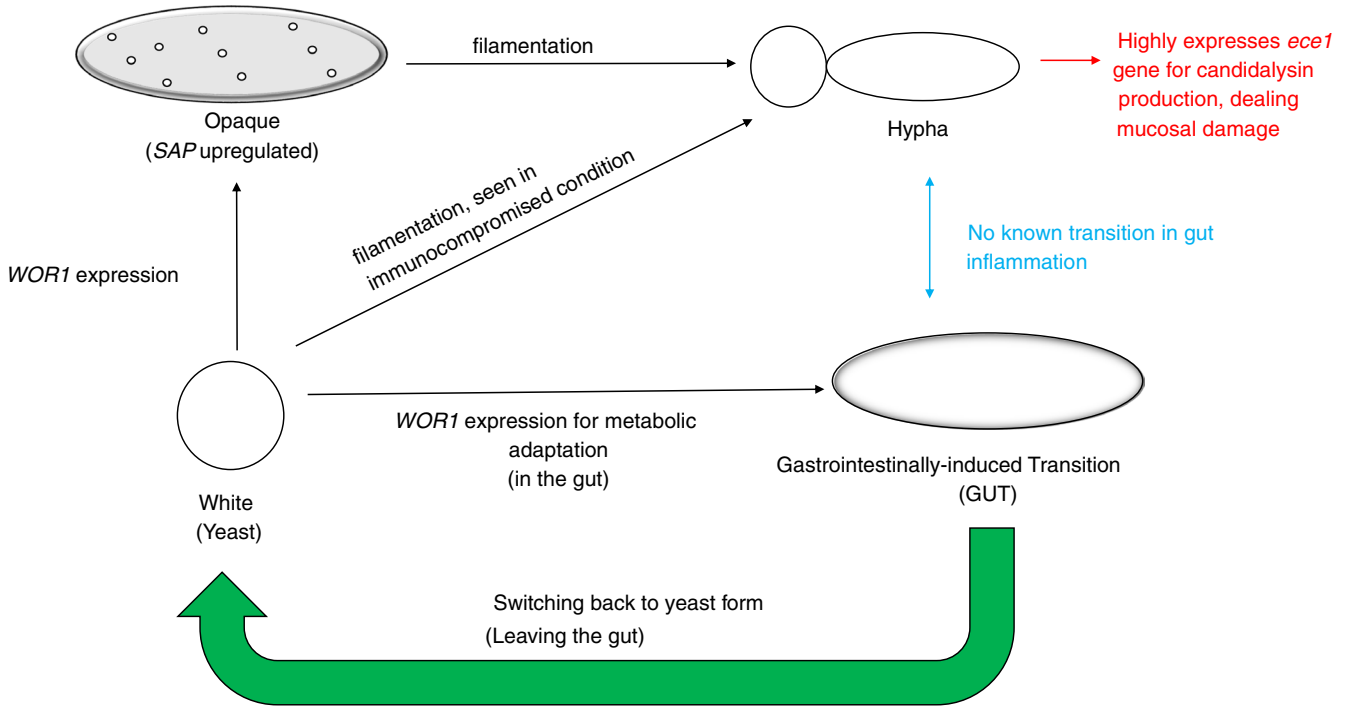

FIGURE 2 *Candida albicans* polymorphic transformation. *C albicans* passage from oral cavity to gastrointestinal tract along with phenotypical transformation via switching from yeast to Gastrointestinal-indUced Transition (GUT) form. While leaving the gut, it switches back to yeast form, suggesting that the GUT form is adaptive to the human gastrointestinal tract milieu in response to indigestible fatty acid, iron and *N*-acetylglucosamine (GlcNAc) present in the distal bowel. While the host switches its immunity into an immunocompromised condition, *C albicans* transforms into its hyphal form that expresses virulence factors and digestive enzymes leading to mucosal damage. Transformation of *C albicans* is linked to immunity conditions and environmental nutrients, but it is not known if *C albicans* induces phenotypical transformation in response to inflammatory condition in the gut

the expansion and reduction of *Candida* and *Saccharomyces* genus respectively.[22,30] Further administration of fluconazole successfully eliminated the over-presented *C tropicalis* and promoted colitis amelioration.[22] In contrast, Tang *et al* showed an opposite result whereby blockade of dectin-1 receptor ameliorated colitis progression.[82] The inactivated dectin-1 reduced dendritic cell signalling for antimicrobial peptide secretion, and eased the inhibitory burden on *Lactobacillus murinus* (*L murinus*).[82] The revived *L murinus* was able to signal dendritic cells to secrete Transforming growth factor beta (TGF-β) and IL-10 for stimulating Treg cells proliferation in mitigating gut inflammation.[82] Distinct results between those of Iliev et al and Tang et al studies might be due to the absence of commensal fungi in Tang's model under specific pathogen-free conditions, where the mice diet (a source of commensal fungi) was sterilised by gamma-ray radiation.[22,82] This was later confirmed by the author, who could not detect any live fungi in the murine faeces[82] (Figure 3).

In addition to dectin-1 signalling, commensal fungi have been found to exacerbate colitis by stimulating host purine metabolism.[99,100] In a wild-type DSS-murine model with robust dectin-1 signalling, daily replenishment of *S cerevisiae* was found to increase intestinal epithelial barrier permeability as well as positively regulate host purine metabolism for uric acid production, which is positively correlated with the severity of colitis by NLRP3 (NALP3) inflammasome binding [99,100]

9 | INTERACTION BETWEEN GUT FUNGI AND OTHER MICROBIAL KINGDOMS IN IBD

9.1 | Polymicrobial biofilm formation

An in vitro model showed that *S aureus* and *C albicans* aggregate and cooperatively form a biofilm, resulting in increased vancomycin resistance and co-infection aided by *C albicans'* hyphae. A positive correlation in the abundance between trans-kingdom species *C tropicalis*, *S marcescens* and *E coli* in CD was observed using Ion Torrent sequencing.[58,101] A subsequent in vitro model further demonstrated the synergy between these three microbes by showing the orchestral effect in forming a polymicrobial biofilm.[58,101] These data highlighted that *Candida* species by themselves may not be the sole contributor to IBD development, but they play a regulatory role in linking microbes from different kingdoms in IBD pathogenesis.

9.2 | Bacteria modulates the active role of gut fungi

How different classes of antibiotics exert inhibitory effect on different bacterial genus spectrum, and how the affected spectrum genus cross-links to the active role of fungi in gut inflammation remains unclear. Two antibiotics, vancomycin and colistin, that target Gram-positive bacteria and Enterobacteriaceae, respectively, have been found to affect the activity of gut fungi and

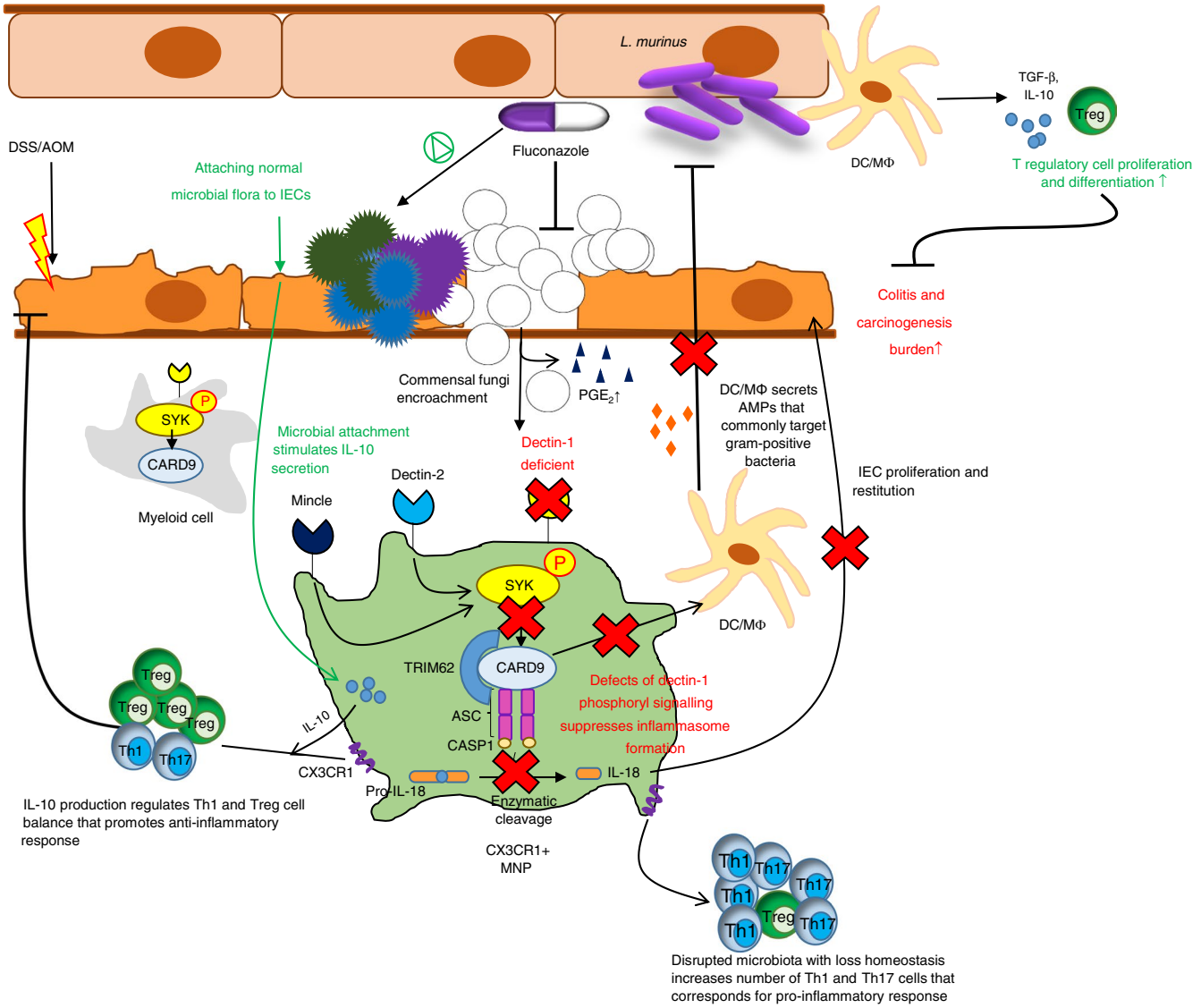

FIGURE 3 Mechanisms of fungal sensing in the host. A robust fractalkine receptor (CX3CR1^{+}) recognises the attachment of normal microbial flora to intestinal epithelial cells (IECs) followed by secretion of interleukin 10 (IL-10), which further balances the T-helper cell 1 (Th1) and regulatory T cells (Treg) number in maintaining host-to-microbe homeostasis. Dextran Sodium Sulfate (DSS) treatment damages IECs and leads to encroachment of commensal fungi. β-glucan in fungal cell wall is recognised by dectin-1 receptor, which signals downstream spleen tyrosine kinase (SYK) - Caspase recruitment domain-containing protein 9 (CARD9) phosphorylation in promoting inflammasome formation and interleukin 18 (IL-18) maturation for IECs reconstitution. Mutant of dectin-1 receptor suppresses the signal for antimicrobial peptides (AMPs) secretion and reduces the inhibitory effect on *L murinus*. The revived *L murinus* stimulates host dendritic cells or macrophages to secrete TGF-β and IL-10, which positively regulate Treg cell proliferation and differentiation in suppressing gut inflammation. Fluconazole inhibits *C albicans* but stimulates drug-resistant filamentous fungi *A amstelodami*, *E nigrum* and *W sebi*, which are recognised by CX3CR1^{+}

modulate the severity of colitis.[102] Vancomycin inhibited Gram-positive bacteria and provided full protection against colitis whereas colistin-treated mice with Enterobacteriaceae depletion were susceptible to colitis, coupled with silenced colitis-modulating functions of gut fungi.[102] Interestingly, when the gut was colonised by *C albicans* and *S boulardii*, Enterobacteriaceae regulated the pathogenic and protective roles of both fungi during the progression of colitis.[102] It was shown that the colonisation level of both fungi was enhanced in the presence of Enterobacteriaceae.[102]

9.3 | Competition between bacteria and fungi

Apart from the synergistic effect between bacteria and fungi, competition is also seen in the gut.[103,104] The commensal bacteria *Bacteroides thetaiotamicron* and *Blautia producta* had a negative correlation in their abundance with *C albicans*.[103,104] Both bacteria activated Hypoxia-inducible factor 1-alpha (HIF-1α), which is a regulator of innate immunity and cathelicidin LL-37 (an antimicrobial peptide) to oppose *C albicans* colonisation.[103,104] Commensal bacteria triggered a host response to oppose *C albicans*

colonisation.[33,105] In a preclinical study using a mice model, the use of *S cerevisiae CNCM I-3856* strain helped prevent adherent-invasive *Escherichia coli* (AIEC) from adhering to an inflamed intestinal mucosa, resulting in the amelioration of colitis.[106]

10 | CONCLUSION

The rapid emergence of IBD worldwide is likely to be the consequence of environmental and genetic influence associated with alterations of the gut microbiota resulting in a dysregulated immune response in the host. Recent fungal sequencing analysis revealed an expansion of the fungi *Candida* and *Malassezia* in the stool and mucosa of IBD patients. Genetic deficiency at the CARD9 loci was also found to be associated with disease severity in IBD. In animal studies, supplementation of *C albicans*, *C tropicalis* (particularly in CARD9 deficient setting) and *S cerevisiae* exacerbates colitis progression whereby one possible mechanism is via increased intestinal permeability and modulated host purine metabolism. In addition to genetic factors, fungal alterations are triggered by environmental factors including diet. Modulation of the fungal microbiota can be considered as a therapeutic opportunity for IBD, as certain strains including *S boulardii*, *S cerevisiae CNCM I-3856 strain* and *S fibuligera* have shown therapeutic effects in human and murine IBD models. Future research should focus on enhancing our understanding on how the fungal microbiota interacts with other components of the gut microbiota and the mechanisms of these interactions, in association with the pathogenesis and development of IBD.

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