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Journal

Journal of Hepatology, 78(4)

ISSN

0168-8278

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Publication Date

2023-04-01

DOI

10.1016/j.jhep.2022.12.006

Peer reviewed

HHS Public Access

Author manuscript

J Hepatol. Author manuscript; available in PMC 2024 April 01.

Published in final edited form as:

J Hepatol. 2023 April ; 78(4): 836–851. doi:10.1016/j.jhep.2022.12.006.

Fungal infections and the fungal microbiome in hepatobiliary disorders

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Summary

Liver and biliary diseases affect more than a billion people worldwide, with high associated morbidity and mortality. The impact of the intestinal bacterial microbiome on liver diseases has been well established. However, the fungal microbiome, or mycobiome, has been overlooked for a long time. Recently, several studies have shed light on the role of the mycobiome in the development and progression of hepatobiliary diseases. In particular, the fungal genus *Candida* has been found to be involved in the pathogenesis of multiple hepatobiliary conditions. Herein, we compare colonisation and infection, describe mycobiome findings in the healthy state and across the various hepatobiliary conditions, and point toward communalities. We detail how quantitation of immune responses to fungal antigens can be employed to predict disease severity, *e.g.* using antibodies to *Saccharomyces cerevisiae* or specific anti-*Candida albicans* antibodies. We also show how fungal products (*e.g.* beta-glucans, candidalysin) activate the host's immune system to exacerbate liver and biliary diseases. Finally, we describe how the gut mycobiome can be modulated to ameliorate hepatobiliary conditions.

Keywords

mycobiome; fungi; liver disease; biliary disease

Introduction

In 2017, 1.5 billion people had cirrhosis and other chronic liver diseases globally, most commonly due to non-alcoholic fatty liver disease (NAFLD, 59%) and hepatitis B virus (HBV, 29%), but also hepatitis C virus (HCV, 9%), alcohol-associated liver disease (ALD, 2%), and others (1%) [1]. Cirrhosis is currently the eleventh most common cause of death

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Authors' contributions

P.H. was responsible for writing the manuscript, BS edited the manuscript.

globally, accounting for two million deaths per year and an estimated 3.5% of global mortality [2–4]. 30,600 persons had gallbladder and other biliary diseases in 2017 [1]. 803,000 persons had liver cancer, and the annual incidence of liver cancer was 953,000 persons in 2017 (HBV 42%, HCV 27%, alcohol use 15%, NAFLD 8%, others 8%) [1].

Hepatobiliary diseases are associated with intestinal bacterial dysbiosis [5], which is defined as an imbalance of bacterial subpopulations and the associated deleterious effects on the colonised host [6, 7]. There are 10^{11} bacterial cells per gram of faeces [8], compared with up to 10^5-10^6 fungal cells [9–12], 10^9-10^{10} virus-like particles[13], or 10^8-10^{10} archaeal cells per gram of faeces [14, 15]. Similar to bacteria, fungi are present at their highest density in the (distal) colon in mice [16] and humans [17]. There are approximately 38×10^{12} total bacterial cells in the human body, which are outnumbered 10-fold by viruses (380×10^{12} in the human body) [18, 19], while there are nearly ~2×10^8 fungal cells, extrapolated from fungal stool estimates [9–11] (Fig. 1A). Further, there are many more bacterial genes in the human gut (at over 22 million [20]) than there are host genes in the entire human body (an estimated 63,000 genes of which 20,000 are protein coding [21]). The exact total number of fungal genes in the gut microbiome is unknown; but we can assume that the total number of fungal genes in the human gut equals at least that of host genes, since important fungal species, such as *Saccharomyces cerevisiae* (*S. cerevisiae*) [22] and *Candida albicans* (*C. albicans*) [23], have at least 6,000 genes each, with others possessing more than 20,000 genes each [24]. Although fungi account for a relatively small proportion of the cell counts and gene numbers of the microbiome, they play a major role in health and disease. The fungal kingdom comprises as many as 6 million species [25], of which approximately 625 have been reported to infect vertebrates and 200 can be human-associated, either as commensals and members of the microbiome or as pathogens that cause infectious diseases [26–28]. Some fungi (such as *C. albicans*, *Blastomyces dermatitidis*, or *Histoplasma capsulatum*) can be dimorphic, *i.e.* they can switch from the unicellular non-invasive yeast morphotype to the multicellular more pathogenic and invasive hyphal morphotype (known as *phase transition*) [29–32]. Fungi are also able to form biofilms, which might make them resistant to antimicrobial agents and difficult to eradicate, *e.g.* in the setting of indwelling medical devices [33]. Fungi are often 100 times larger and 10 times longer than bacteria [9, 10], typically averaging 2–25 μm in length [34, 35] *vs.* 2–3 μm for bacteria [36], 20–200 nm for viruses [36], 1–5 μm for archaea [37, 38], and 10–30 μm for human cells [36] (Fig. 1B). This size difference indicates the relatively large biomass that fungi contribute to the gut microbiome despite being present at lower cell counts than bacteria [28].

Culture-based methods are good at detecting the dominant fungal species but neglect fungal communities that are less abundant and cannot be cultured. Culture-independent approaches are used to determine fungal diversity including high-throughput sequencing methods targeting 18S, internal transcribed spacer (ITS) 1, ITS2, and 26S/28S of fungal ribosomal RNA (rRNA), as well as whole-genome shotgun sequencing [39, 40]. It has been reported that ITS target regions outperform 18S and 26S rRNA regions, and ITS2-based sequencing outperforms ITS1-based sequencing by more accurately identifying key fungal taxa [40]. However, all current methods have some bias in their detection, *e.g.* ITS1 primers favour amplification of basidiomycetes and ITS2 primers are biased toward ascomycetes [41]. Hence, to reduce this bias, it has been suggested to use combinations of different

primer pairs or to analyse different parts of the ITS region in parallel (*e.g.* with ITS1, ITS2, ITS3, ITS4 primers) [41]. The findings could further be confirmed by quantitative PCR (qPCR) or culture in the appropriate context. Nevertheless, most studies rely on only one methodology at a time.

This review will compare colonisation and infection, describe changes of the mycobiome in patients with liver and biliary diseases, and will detail mechanisms by which fungi can contribute to hepatobiliary diseases.

The mycobiome in healthy individuals

The Human Microbiome Project demonstrated that fungal diversity is significantly lower than bacterial diversity in the human gut and that the human mycobiome exhibits high inter- and intraindividual variability [42]. Nevertheless, a core gut mycobiota has been found comprising *Candida* (especially *C. albicans*), *Saccharomyces* (in particular *S. cerevisiae*), *Penicillium*, *Aspergillus*, *Cryptococcus*, *Malassezia* (particularly *Malassezia restricta*), *Cladosporium*, *Galactomyces*, *Debaryomyces* and *Trichosporon* [9, 43]. Of note, only a small number of fungi are able to colonise the gut (including *Candida* and *Galactomyces*); however, other fungi derived from dietary (such as *S. cerevisiae*) or environmental (*Aspergillus* species) sources are detectable and are likely to impact gut ecology [44]. Gut mycobiota are established via mother-to-offspring transfer in early life [45]; transfer can also occur via vaginal delivery (*e.g. C. albicans*) [46] or via saliva or skin contact (*e.g. Malassezia*) [47]. There is strong evidence that the gut mycobiome is dynamically shaped by nutrition. A vegetarian diet is associated with a significantly increased relative faecal abundance of *Candida* spp. in humans, whereas an animal-based diet is associated with significantly increased *Penicillium* and decreased *Debaryomyces* and *Candida* spp. [48]. The gut mycobiome is also affected by drug therapy, geographical location, oral hygiene [49], ethnicity, urbanisation, and lifestyles [50]. Short-chain fatty acids have been found to inhibit the growth of *C. albicans* through the stimulation of intestinal mucosal immunity [50]; *Aspergillus* also correlates negatively with short-chain fatty acid content in the diet [51]. Notably, fungi are able to synthesise B vitamins and vitamin D and can thereby impact and shape the host's immune system [49].

Colonisation *vs*. infection

Colonisation occurs when the presence and multiplication of a microbial agent on a body surface does not cause a specific immune response or infection in the host [52, 53]. That said, healthy humans are known to produce antibodies against various colonising microbes including *C. albicans* [54–56], so the aforementioned definition might have to be adapted to include the term '*clinically apparent* immune response'. However, when the relationship between the agent and the host changes, *e.g.* when the normal microbiota of the gastrointestinal tract enters the bloodstream in the setting of microbial translocation, infection can result [52]. Infection refers hereby to invasion of the body tissue by harmful/pathogenic microorganisms resulting in disease [57]. Clinically, an infection is often associated with fever, tachycardia, leucocytosis, increased inflammatory markers (*e.g.* C-reactive protein or procalcitonin) and/or a positive blood culture [58], which can further help

to delineate it from colonisation. A fungal infection can also be asymptomatic, particularly when it is superficial.

Fungal infections are common with around 1 billion people estimated to have skin, nail, and hair fungal infections worldwide (primarily *Trichophyton rubrum* [*T. rubrum*], *T. interdigitale* [*mentagrophytes var. interdigitale*], *Microsporum canis* [*M. canis*], *M. audouinii*, *T. tonsurans* and *T. verrucosum* [59]), while around 134 million females have recurrent vulvovaginal candidiasis globally [60], although these infections are usually not associated with mortality. Mortality due to fungal infections occurs mostly in immunocompromised patients and roughly 90% of deaths related to fungal infections are due to species of *Aspergillus*, *Cryptococcus*, *Candida*, or *Pneumocystis*, as well as *Coccidioides* and *Histoplasma*, which can infect even immunocompetent hosts in endemic regions [26]. The World Health Organization recently published a list of 19 fungal pathogens, its first fungal priority pathogens list, for which major treatment and management challenges exist, such as drug resistance [61]. The critical priority group of the fungal priority pathogens list includes *Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus*, and *C. albicans* [61].

Some fungal populations (including *C. albicans*) can be commensals during health but can invade and infect the host when gut barrier function is disrupted and/or the host becomes immunocompromised [43]. Fungal virulence factors promoting an infection *vs.* sole colonisation can include a yeast-to-hypha dimorphic transition with a hyphae-associated genetic programme, which enables adhesion, active invasion, micronutrient acquisition (including thiamine, pyridoxine, and nicotinic acid), direct host cell damage, biofilm formation, and various types of immune evasion [62]. Secretion of certain molecules (such as proteases, phospholipases, and lipases) advances fungal adhesion and invasion [62, 63]. Further, a capsule can decrease host immune responses by downregulating inflammatory cytokines, depleting complement components, and inhibiting the antigen-presenting capacity of monocytes, preventing phagocytosis and facilitating infection [64]. Another virulence factor is production of pigments, *e.g.* melanin, to protect the fungus against oxidative stress [65]. Moreover, fungal high-frequency antigenic variation provides a way for a number of fungi, including *Pneumocystis*, to survive an attack by the adaptive immune system [66]. Interestingly, some fungi (*e.g. Rhizopus oryzae*, the most common cause of mucormycosis) can invade blood vessels and use haemoglobin as an iron source [67].

Inter-kingdom relationship between fungi and bacteria

Antibiotics can alter the gut mycobiome [68, 69] and conversely, antifungals can alter the bacterial microbiome [70, 71]. Fungi and bacteria interact with each other. The persistent biological interactions, or symbiosis, between fungi and bacteria encompass mutualism, neutralism, competition, commensalism, and parasitism [72]. Mutualism refers to a state where both microorganisms derive a benefit from their relationship, neutralism where there is no effect on both microorganisms, competition where both derive a harm, commensalism where one microorganism derives a benefit with no effect on the other, and parasitism where one derives a benefit at the cost of another microorganism [9]. An apparent mutualism is present between *Candida tropicalis* and the bacterial species *Serratia marcescens* and

Escherichia coli in Crohn's disease, where the three species appear to interact in a biofilm, which is thicker than the ones produced by the species separately [73]. Competition has been shown in a study in which the nematode *Caenorhabditis elegans* was co-infected with *C. albicans* and *Acinetobacter baumanii* (*A. baumannii*), where *A. baumannii* inhibited filamentation and attenuated the virulence of *C. albicans*. However, *C. albicans* itself also inhibited growth of *A. baumannii* via the quorum-sensing molecule farnesol [74]. Commensalism is exemplified by *Helicobacter pylori* that can enter and survive in *C. albicans* vacuoles in the stomach without an apparent benefit for *C. albicans* [75]. Parasitism can be observed between *C. albicans* and *Clostridium difficile* (*C. difficile*), where the presence of *C. albicans* allows *C. difficile* to grow under (normally toxic) aerobic conditions, whereas *C. difficile* via its fermentation product *p*-cresol inhibits hypha and biofilm formation and virulence of *C. albicans* [76]. This multitude of interactions underlines the complex interactions between fungi and bacteria.

Faecal mycobiome in liver and biliary diseases

Changes in the mycobiome have been observed in essentially all hepatobiliary diseases, often associated with an increased abundance of the genus *Candida* and the species *C. albicans*.

Primary sclerosing cholangitis

The mycobiome in primary sclerosing cholangitis (PSC) is characterised by altered composition and increased alpha diversity [77], with a decrease in *S. cerevisiae* and an increase in the genera *Exophiala* [77], *Candida*, *Humicola*, and the species *Humicola griseum* [78] (Table 1, Fig. 2). *S. cerevisiae* has anti-inflammatory properties and was shown to be reduced in patients with inflammatory bowel disease flares [79]. Patients with PSC and biliary *Candida* infection (most commonly *C. albicans*) have more severe cholangitis with higher C-reactive protein and serum bilirubin levels compared to those without *Candida* infection [80]. Further, biliary *Candida* infection (detectable in five out of 55 patients) was associated with reduced survival in patients with PSC, whereas bacterial infections of the biliary system (present in 41 out of 55 patients) did not affect survival [81]. This indicates that biliary *Candida* infection is associated with poor prognosis and that these patients might be considered for liver transplantation [81]. In particular, patients with PSC and persistent biliary candidiasis show significantly reduced transplantation-free survival and elevated cholangiocarcinoma incidence, whereas survival of patients with transient biliary candidiasis is comparable to that of candidiasis-free patients [82].

Primary biliary cholangitis

Although various studies demonstrate bacterial microbiome changes in primary biliary cholangitis (PBC), including increased relative abundance of *Streptococcus* [83–85], *Veillonella* [83, 84, 86], and decreased *Faecalibacterium* [84, 85], no faecal mycobiome studies have been carried out in patients with PBC to date. There are case series in which patients with PBC succumbed to fungal infections (*Pneumocystis* spp., mucormycosis [87], or urosepsis related to *Candida* spp. [88]). However, there is no evidence yet that the fungal microbiome is involved in the pathogenesis of PBC.

Gallbladder disease

Several reports have shown that *Candida* spp. (especially *C. albicans*) could be cultured from bile or from gallbladder tissue from patients with cholecystitis or common bile duct obstruction [89]. This likely reflects a fungal infection, as there is no evidence in the literature to show fungal colonisation of biliary tissue in healthy individuals. *Candida* spp. and *C. albicans*-positive bile cultures have a negative prognostic value in multiple diseases affecting the biliary system; in particular, a *Candida*-positive acute acalculous cholecystitis (five out of five infected patients in intensive care died) and malignant biliary obstruction (five out of 12 patients died) are associated with high mortality [90]. In another study, the bile of 36 patients with moderate and severe acute cholecystitis was analysed and the bile from 31 of these patients contained bacteria and/or fungi, as determined by ITS2 sequencing (six samples contained fungi, including three cases of *C. albicans*, one of *C. humilis*, and two of *S. cerevisiae*, of which only one case of *C. albicans* was detected by culture) [91]. Culture identified only 40 (38%) of the 106 microbes identified by sequencing [91]. It is known that patients are at increased risk of postoperative gastrointestinal comorbidities, such as colorectal cancer, following cholecystectomy [92, 93]. It was speculated that cholecystectomy may have an impact on intestinal microbial homeostasis, which may facilitate colorectal carcinogenesis and progression [94]. And, indeed, increased relative faecal abundance of *C. glabrata* and unassigned *Aspergillus,* and decreased *C. albicans* have been observed in patients following cholecystectomy (compared to controls) [92]. Of note, patients who underwent cholecystectomy and had pre-cancerous colonic lesions (low- and high-grade intraepithelial neoplasia) or colorectal cancer had higher levels of *C. glabrata* than patients who underwent cholecystectomy but had no (pre-)cancerous lesions [92]. Interestingly, when gallstones were analysed after cholecystectomy, no fungal DNA was detected in pigmented and cholesterol gallstones [95].

Alcohol-associated liver disease

Candida spp. and *C. albicans* are increased in all mycobiome studies investigating patients with alcohol-associated liver disease (ALD) [55, 96–98]. Two studies in ALD used ITS1-sequencing: one found that patients with alcohol-associated hepatitis (AH) and alcohol use disorder (AUD) had increased faecal proportions of the genus *Candida* and decreased *Penicillium*, *Saccharomyces*, and *Debaryomyces* in relation to controls [96], whereas the other study demonstrated that a group of patients with AUD, AH, and alcohol-associated cirrhosis had high faecal proportions of *Candida* and low concentrations of *Epicoccum* and *Debaryomyces* compared with controls [97]. Another study employed qPCR and culture methods and demonstrated that patients with AH had a significantly higher faecal fungal load and *C. albicans* abundance than controls and patients with AUD [98]. Patients with AUD have increased faecal proportions of the genera *Candida*, *Debaryomyces*, *Pichia*, *Kluyveromyces*, *Issatchenkia*, *Scopulariopsis* and species *C. albicans*, *C. zeylanoides*, *Issatchenkia orientalis*, *Scopulariopsis cordiae*, and a decrease in the relative abundance of *Aspergillus* spp. and *Kazachstania humilis* per ITS2-sequencing [55]. Interestingly, the presence [99] and higher relative faecal abundance of *Malassezia restricta* [55] was associated with more severe liver disease in patients with AUD. Only two weeks of abstinence was sufficient for the improvement of liver cell necrosis and apoptosis marker caspase-cleaved and intact cytokeratin 18 (CK18-M65) levels and controlled attenuation

parameter (CAP) per fibroscan [55]. This was associated with significantly reduced proportions of the genera *Candida*, *Malassezia*, *Pichia*, *Kluyveromyces*, *Issatchenkia*, *Claviceps*, *Cyberlindnera*, and *Hanseniaspora* and lower proportions of the species *C. albicans*, *C. zeylanoides*, *Malassezia restricta*, *Issatchenkia orientalis*, and *Cyberlindnera jadinii* compared with the period prior to abstinence [55]. These findings indicate that fungal dysbiosis is at least partially reversible after abstinence. Patients with AUD had significantly increased serum anti-*C. albicans* IgG and IgM titres compared to controls, and anti-*C. albicans* IgG titres decreased after abstinence [55].

Non-alcoholic fatty liver disease

ITS2-sequencing showed that the more severe forms of non-alcoholic fatty liver disease (NAFLD), namely non-alcoholic steatohepatitis (NASH) and NAFLD with more advanced fibrosis stages (F2-F4), are associated with high faecal amounts of *C. albicans*, *Pichia barkeri*, *Mucor* spp., and *Cyberlindneria jadinii* compared with non-alcoholic fatty liver (simple steatosis) and NAFLD with no/mild fibrosis (F0-F1) [56]. These advanced forms also have higher faecal log ratios of *Mucor* spp./*S. cerevisiae* and *Babjeviella inositovora*/*S. cerevisiae* compared with controls [56]. It is important to note that the majority of the differences in the mycobiome between early and advanced NAFLD were observed in lean rather than obese individuals, as no significant differences between early and advanced NAFLD were noticed when only obese subgroups were compared [56]. You *et al*. found that the relative faecal abundances of the genera *Talaromyces*, *Paraphaeosphaeria*, *Lycoperdon*, *Curvularia*, *Phialemoniopsis*, *Paraboeremia*, *Sarcinomyces*, *Cladophialophora*, and *Sordaria* were higher, whereas the relative abundances of the genera *Leptosphaeria*, *Pseudopithomyces*, and *Fusicolla* were decreased in patients with NAFLD relative to controls [100]. The same study found that the genera *Paramycosphaerella*, *Fusicolla*, *Arthrinium*, *Triparticalcar*, *Trichoderma*, and *Cladosporium* are increased in patients with NASH *vs*. non-alcoholic fatty liver; and *Cladosporium*, *Staphylotrichum*, *Paecilomyces*, and *Thermomyces* were increased in patients with NAFLD F2-F4 *vs*. NAFLD F0-F1 [100]. When the faecal mycobiome of advanced fibrosis stages between NAFLD and ALD are compared, patients with NAFLD F3-F4 have significantly higher faecal proportions of *Mucor* spp. and lower proportions of *Candida* spp., *C. albicans*, *Debaryomyces* spp., and *Blumeria* spp. than patients with ALD F3-F4 [56]. Besides other metabolic conditions, type 2 diabetes mellitus (T2DM) is associated with NAFLD [7]. In patients with NAFLD and T2DM, the proportion of *C. albicans*, *Pichia barkeri*, *Malassezia* spp. was increased while that of *Kazachstania* spp. and *Blumeria* spp. was reduced in comparison to patients with NAFLD without T2DM [56]. Patients with T2DM were found to have higher faecal proportions of the genera *Candida* [101, 102], *Cladosporium*, *Kodamaea*, *Meyerozyma*, *Mortierella* [102], and *Malessezia furfur* [103], and depleted faecal *Issatchenkia*, *Macrophomina*, *Marasmius*, *Gymnopilus*, *Saccharomyces*, *Trichoderma*, *Cochliobolus*, *Psathyrella*, and *Clavispora* compared to controls [102].

Viral hepatitis

HBV- and HCV-infected patients exhibit increased translocation of fungal products into the serum, as demonstrated by elevated fungal beta-glucan serum levels compared with controls [104, 105]. Patients with chronic HBV-hepatitis or with HBV-cirrhosis are more likely to

test positive for *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *S. cerevisiae* in their stool, and they also have higher faecal DNA levels of *C. albicans*, *C. parapsilosis*, *C. krusei*, and *S. cerevisiae* compared with controls [106]. Chen *et al.* found that patients with HBV infection and HBV-related liver disease more frequently test positive for *Aspergillus* spp. (including *A. versicolor*), *Candida* spp. (including *C. albicans* and *C. tropicalis*), *Saccharomyces* spp. (including *S. cerevisiae*), and *Simplicillium* spp. in their faeces than controls [107]. Using culture methods, more colony-forming units of faecal *Saccharomyces* were detected in stool from patients with chronic hepatitis B, including those with related cirrhosis, compared to controls [108]. Patients with chronic HCV infection, including those with cirrhosis, have a significantly higher faecal fungal load and test positive for *Candida* spp. more frequently than controls [109].

Cirrhosis

It is well known that patients with cirrhosis have a profoundly dysbiotic bacterial microbiome [6, 110]. Patients with cirrhosis secondary to diverse aetiologies have low faecal bacterial and fungal Shannon diversity indices compared with controls, and those indices correlate indirectly with model for end-stage liver disease (MELD) scores [111]. Those patients with cirrhosis requiring hospital admission also have higher relative faecal abundance of *Candida* than outpatients with cirrhosis or controls [111]. Another study used qPCR and culturing techniques and showed higher detection of *Candida* in duodenal fluid samples from patients with cirrhosis compared with controls (qPCR 81.5% *vs.* 61.5%, culture 66.7% *vs.* 46.2%), although this did not reach statistical significance [112]. Mucosal infection with *Candida* occurs in cirrhosis and correlates with disease severity: oesophageal candidiasis was diagnosed in 100 of 2,762 patients with cirrhosis, and patients with oesophageal candidiasis had higher MELD scores (12.4 *vs.* 11.2, $p = 0.007$) and were more likely to develop acute-on-chronic liver failure (26% *vs.* 10%, $p = 0.003$) than patients with cirrhosis without oesophageal candidiasis [113]. Fungal infections in patients with cirrhosis, mainly caused by *Candida* spp., are often associated with delayed diagnosis, higher rates of acute-on-chronic liver failure, inpatient stay, intensive care unit admissions, and worse 30-day survival than no infection or bacterial infections [114, 115]. The case fatality rate was 30% with most fungal infections but >50% for fungemia and fungal peritonitis [115]. Similarly, bacterial and fungal infections related to liver transplantation are common, occurring in more than half of patients, mainly due to the complex surgical procedures [116]; in this context invasive fungal infections are associated with high mortality rates ranging from 25% to 67% [117].

Hepatocellular carcinoma

Aflatoxins, food contaminants produced by the *Aspergillus* spp. including *Aspergillus flavus* and *Aspergillus parasiticus*, are known human carcinogens that have been shown to be causative agents in the pathogenesis of hepatocellular carcinoma (HCC) [118]. Aflatoxin B1 (AFB1) is the most potent known hepatocarcinogen [118]. In a case-control study with 348 Chinese patients with newly diagnosed HCC and 597 controls without liver disease, patients with HCC had higher AFB1 exposure than the control group (odds ratios [OR] = 6.49 and 6.75 for exposure years and exposure levels, respectively) [119]. AFB1-related HCC is primarily found in Southeast Asia and Sub-Saharan Africa [120], although elevated

AFB1 DNA adducts were also occasionally detected in patients with HCC in the Southern United States [121]. Further, genetic susceptibility may also play a role in AFB1-related HCC. Individuals with rs#7003908 G alleles in the X-ray repair cross-complementing group 7 (*XRCC7*) gene (XRCC7-TG/-GG) were at significantly higher risk of AFB1-related HCC (OR 3.45 and 5.04) than rs#7003908 T homozygotes (XRCC7-TT) [119].

Although patients with cirrhosis already have elevated faecal levels of *Candida* [111], patients with HCC were found to have even higher faecal proportions of *Candida* and *C. albicans* than patients with cirrhosis, but lower proportions of the genera *Kazachstania*, *Debaryomyces*, *Xeromyces*, *Amorphotheca*, and *Blastobotrys* [122]. In a mouse model of HCC, gavage with *C. albicans* resulted in exacerbated HCC volume, which was dependent on the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 6 (NLRP6) inflammasome [123]. Moreover, development of HCC in a patient with cirrhosis increases their risk of oesophageal candidiasis (OR 10.04) [113].

Immune response to fungal antigens in hepatobiliary diseases

- Antibodies to *S. cerevisiae*—Antibodies to *S. cerevisiae* (ASCA) detect *S. cerevisiae* mannan, a cell wall carbohydrate that is common to most fungi [124]. ASCA also cross-react with mannan from other fungal species including S*. boulardii*, *S. pastorianus*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, and *C. albicans* [125]. In particular, *C. albicans* is known to be a strong immunogen for ASCA formation [126]. Patients with PSC, and those with anti-mitochondrial antibody-negative or -positive PBC show higher ASCA prevalence (44%, 53%, and 18%, respectively) than blood donors (5%), although the presence of ASCA was not associated with any clinical or biochemical parameters [127]. Both ASCA IgA and IgG positivity were higher in these conditions than in controls [127, 128]. In PBC, ASCA titres correlate with elevated levels of circulating IgA, which may be an indirect sign of enhanced mucosal immunity [127]. Serum ASCA-IgG titres are higher in patients with alcohol-associated cirrhosis *vs*. controls and *vs*. patients with HBV-cirrhosis [97]. Higher ASCA-IgG titres are associated with decreased 5-year survival of patients with alcohol-associated cirrhosis [97]. Serum ASCA-IgG levels are also higher in patients with AH than in those with AUD or controls [96]. Moreover, increased ASCA-IgG titres predict worse 90-day and worse 180-day survival in patients with AH [96]. A model consisting of the ASCA-IgG titre and MELD score has a significantly better diagnostic performance for mortality than the MELD score alone [96].

- Specific anti-*C. albicans* antibodies—Specific serum anti-*C. albicans* IgG and IgM titres are increased in patients with AUD *vs*. controls, and interestingly, the anti-*C. albicans* IgG titre (but not the IgM titre) decreases significantly in patients after abstinence [55]. These changes parallel elevated faecal proportions of *C. albicans* in patients with AUD (*vs*. controls), which decrease after abstinence [55]. Similarly, plasma anti-*C. albicans* IgG titres are increased in patients with NAFLD and advanced fibrosis (F3-F4) *vs*. patients with NAFLD and no/early fibrosis (F0-F2) and *vs*. controls, and anti-*C. albicans* IgG titres correlate with the faecal *C. albicans*/*S. cerevisiae* log ratio [56]. Anti-*C. albicans* IgG titres hence correlate with disease activity in ALD [55] and NAFLD [56], indicating more systemic exposure to *C. albicans* in more severe liver disease.

- Immune response to candidalysin—Candidalysin is a secreted cytolytic peptide toxin from *C. albicans* that directly damages epithelial membranes, triggers a danger response signalling pathway and activates epithelial immunity [129]. *C. albicans* strains lacking this toxin do not activate or damage epithelial cells and are avirulent in animal models of mucosal infection [129]. The extent of cell elongation 1 (*ECE1*) gene encoding candidalysin was more frequently present in the stool of patients with AH than AUD [98]. Further, mice gavaged with wild-type ECE1-positive *C. albicans* had significantly higher serum alanine aminotransferase (ALT) levels, hepatic triglycerides and inflammation than mice gavaged with ECE1-negative *C. albicans* in a 2-week ethanol binge model, supporting the notion that ECE1-positive *C. albicans* exacerbates ethanol-induced steatohepatitis in mice [98]. Similarly, ECE1/candidalysin-positive patients with AH have significantly higher MELD scores and 90-day mortality rates than ECE1-negative patients [98]. Secretory IgA plays an important role in gut barrier protection and *C. albicans*-induced secretory IgA preferentially binds *C. albicans* hyphae (fungal morphotype associated with virulence) over its yeast morphotype [130]. Secretory IgA also binds ECE1-derived candidalysin, though this binding is reduced in patients with Crohn's, indicating fungi-related immune dysregulation [130]. It is possible that this aberrant immune response also plays a role in ALD.

Mechanisms by which fungi contribute to hepatobiliary disease

Antifungal treatment improves liver disease in various mouse models, including ethanol- and western diet-induced steatohepatitis [56, 97, 131]. Further, colonisation with *C. albicans* [98, 123] or *Malassezia restricta* [99] exacerbates liver disease. Therefore, fungi and their products contribute to liver disease. In the following sections, we will discuss possible mechanisms by which fungi could contribute to the development and progression of liver disease.

Pathways by which the immune system recognises fungi and mounts an immune response

Pattern recognition receptors on immune cells can sense pathogen-associated molecular patterns on fungi [132] and initiate an immune response. The receptor groups include C-type lectin receptors, including the mannose receptor, CLEC7a (=Dectin-1), CLEC4n (=Dectin-2), Mincle, DC-SIGN; Toll-like receptors (TLRs), including TLR2, TLR3, TLR4, TLR6, TLR7, TLR9; nucleotide-binding oligomerization domain-like receptors (NLRs), including NLRP3, NLRP4, NLRP6, NLRP10, nucleotide-binding oligomerization domain-containing protein 1 and 2; and galectin 3 [132, 133]. Once these receptors recognise a fungus, they trigger signalling cascades (such as the SYK-CARD9, RAF, MYD99, TRIF pathways) to produce cytokines, *e.g.* interleukin 1β (IL-1β), IL-6, IL-12, IL-23, transforming growth factor-β and interferon-γ, which induce IL-17A-producing T helper (Th)17 and Th1 cells [9]. This inflammatory response can contribute to hepatobiliary disease.

Beta-glucans

Chronic alcohol administration increases mycobial populations and the translocation of fungal beta-glucan into the systemic circulation in mice [97, 131]. Oral administration of the

antifungal amphotericin B reduces faecal fungal overgrowth and beta-glucan translocation [97]. Antifungal treatment with oral amphotericin B or caspofungin prevents ethanol-induced liver disease in mice without changing plasma bacterial lipopolysaccharide levels [97, 131]. Similarly, the faecal abundance of bacterial subpopulations does not change after treatment with antifungals in mouse models of ethanol-induced [97] and western diet-induced steatohepatitis [56]. Beta-glucan induces liver inflammation via CLEC7a on Kupffer cells, as shown in experiments employing bone marrow chimeric mice [97]. CLEC7a-dependent activation of caspase-1 via NLRP3 [134, 135] leads to increased inflammatory IL-1β expression and secretion, which subsequently contributes to hepatocyte damage and ethanol-induced liver disease [97]. CLEC7a also plays a role in diet-induced steatohepatitis, since its hepatic expression is significantly increased in patients with NASH and mice on a high-fat diet, whereas Clec7a-deficient mice and mice treated with a Clec7a-antagonist are protected from diet-induced steatohepatitis and fibrosis [136].

Candidalysin

Rats infected with *C. albicans* by intraperitoneal injection develop hepatic steatosis, increased serum ALT levels, inflammatory markers, and pronounced lipid peroxidation [137]. This raises the question of how *C. albicans* causes liver disease. One effector could be its secreted cytolytic toxin candidalysin. Candidalysin exacerbates ethanol-induced liver disease and is associated with increased mortality in mice [98]. Candidalysin does this independently of Clec7a on bone marrow-derived cells, since mice that were transplanted with bone marrow-derived cells from Clec7a-deficient mice and subsequently gavaged with wild-type candidalysin-positive *C. albicans* had significantly higher serum ALT levels, hepatic triglycerides and inflammation than the same chimeric mice that were gavaged with candidalysin-negative *C. albicans* [98]. Candidalysin can damage primary hepatocytes in a dose-dependent manner *in vitro* and is associated with liver disease severity and mortality in patients with AH [98]. *C. albicans* is the major fungal inducer of human Th17 cell antifungal responses [138]. Th17 cells appear to play a role in PSC, as stimulation of peripheral blood mononuclear cells (PBMCs) with *C. albicans* results in significantly higher rates of Th17 cells in PSC-PBMCs than in PBMCs from healthy controls [139]. *C. albicans* strains with high immune-cell-damaging capacity (HD strains) were discovered in patients with ulcerative colitis and these HD strains aggravated intestinal inflammation *in vivo* through IL-1β-dependent mechanisms [140]. Th17 cell antifungal responses by HD strains in the gut were dependent on candidalysin [140]. It is possible that a similar mechanism might also contribute to liver disease, although candidalysin did not alter gut barrier function in the aforementioned murine ethanol-induced liver disease model [98]. Candidalysin activates the NLRP3 inflammasome [141]. The NLRP3 inflammasome is known to play a central role in the development of NAFLD/NASH [142–144]. It is hence reasonable to hypothesise that Candidalysin also contributes to the pathogenesis of NAFLD/NASH. Similarly, the *C. albicans*-induced exacerbation of HCC in a mouse model was shown to be NLRP6 inflammasome-dependent [123].

Prostaglandins

Prostaglandins are known to play a role in liver disease. In particular, prostaglandin E2 (PGE2), plays a pivotal role during inflammatory processes [145]. Expression of the key

enzymes of PGE2 synthesis, cyclooxygenase 2 and microsomal PGE synthase 1, are increased in human NASH livers compared with controls and correlate with the NAFLD activity score [145]. PGE2 drives immunosuppression in patients with acute decompensation of cirrhosis and end-stage liver disease, partially by suppressing macrophage cytokine secretion and bacterial killing, worsening outcomes [146]. Fungi were found to participate in that pathogenic process. The PGE2-producing fungus *Meyerozyma guilliermondii* was increased in mice with ethanol-induced steatohepatitis [131]. Further, supplementation with *Meyerozyma guilliermondii* worsens ethanol-induced liver disease in amphotericin-treated mice, by increasing production of PGE2; liver disease was reduced by concurrent administration of the cyclooxygenase 2-inhibitor indomethacin in this mouse model [131]. Concurrent administration of an antifungal also abrogates PGE2 formation and ethanol-induced liver disease [131]. Moreover, *C. albicans* is a potent inducer of the Th17 response via PGE2; PGE2 is induced by the *C. albicans* components mannan and β-glucan that are recognised by the mannose receptor and the Clec7a/Tlr2 pathway, respectively [147, 148]. Th17 cells are known to worsen liver disease by inducing inflammation and fibrosis via Kupffer cells and hepatic stellate cells, respectively, and injure hepatocytes via IL-17 [149]. Th17 cells also worsen biliary inflammation in PBC [150, 151]. Further, *C. albicans* has evolved the capacity to produce PGE2 from arachidonic acid to promote its own colonisation in the host gut [152–154]. *C. albicans* mutants lacking PGE2 production (genetically missing *ole2*, a fatty acid desaturase) are unable to colonise the murine gastrointestinal tract, which is improved by PGE2 supplementation [152]. However, the *C. albicans* mutant did not affect survival in a murine model of systemic candidiasis nor did it change infection of the tongue or vaginal tissue in mouse models of oropharyngeal and vulvovaginal candidiasis [152].

Aflatoxins

Aflatoxins, produced mainly by *Aspergillus* spp., give rise to the development of HCC by inducing DNA strand breaks, oxidative stress, adduct formation, and gene mutations [118]. AFB1 induces persistent single- and double-strand DNA breaks [155, 156]. It induces oxidative stress and lipid peroxidation [157–159]. AFB1 interacts with DNA and forms DNA adducts, such as 2,3-dihydro-2-(N7-guanyl)-3-hydroxy-aflatoxin B1 [160, 161]. AFB1-DNA adducts negatively correlate with expression and polymorphisms of ADAMTS5 (ADAM metallopeptidase with thrombospondin type 1 motif 5) [162], which is a tumour suppressor gene that inhibits tumour angiogenesis and metastasis [163]. Lost expression of ADAMTS5 protein is associated with progression of HCC and poor prognosis [164]. The metabolically active form of AFB1 can cause gene mutations with a preference for GC to TA transversions [165]. Similarly, it is well known that AFB1 induces the transversion of G->T in the codon 249 of the p53 tumour suppressor gene, which has been referred to as a molecular marker for HCC due to AFB1 [166–170]. AFB1 also contributes to HCC formation by activating oncogenes including N-ras, c-Ha-ras, and c-Myc [170–173]. There is a synergistic effect of HBV and AFB1 exposure on the development of HCC. The relative risk of HCC development for HBV infection alone was 7.3, for AFB1 exposure alone 3.4, and for HBV and AFB1 combined 59.4 [174]. There are several explanations for this: chronic HBV infection induces cytochrome P450s that metabolise inactive AFB1 to the mutagenic AFB1–8,9-epoxide; chronic HBV induces hepatocyte necrosis and increases

reactive oxygen and nitrogen species levels, increasing the likelihood of AFB1-induced p53 249ser mutations; and nuclear excision repair to remove AFB1-DNA adducts is inhibited by HBV X protein [175]. Another naturally occurring mycotoxin is fumonisin B1 produced by *Fusarium verticillioides*, which has been shown to induce hepatocellular and cholangiocellular tumours with malignant potential [176].

Therapeutic modulation of the mycobiome in hepatobiliary disease

Manipulation of the gut mycobiome has shown promise in various experimental models of hepatobiliary disease. Although antifungal treatment itself can cause significant hepatotoxicity and even death [177, 178], various rodent studies have demonstrated that oral administration of the antifungals amphotericin and caspofungin can improve ethanol-induced liver disease [97, 131] or diet-induced steatohepatitis [56]. Whereas gavage with *C. albicans* [98] or *Meyerozyma guilliermondii* [131] exacerbates experimental ethanol-induced liver injury, gavage with other fungi improves various experimental liver diseases. *S. boulardii* is the most studied yeast probiotic [179]. Supplementation with *S. boulardii* can ameliorate forms of experimental acute liver injury: it attenuates clarithromycin- and methotrexate-induced hepatic lipid peroxidation and depletion of the antioxidant glutathione [180]. *S. boulardii* also improves D-galactosamine-induced liver injury in mice, lowering liver transaminase levels and alleviating hepatocyte necrosis, haemorrhage and inflammatory infiltration on histology [181]. Supplementation with *S. boulardii* further ameliorates experimental metabolic liver disease: it attenuates hepatic steatosis, inflammation, and fat mass in *db*/*db* mice [182]; hyperglycaemia, dyslipidaemia, and liver inflammation in streptozotocin-diabetic mice [183]; as well as liver injury, inflammation, steatosis, and fibrosis in mice with methionine-choline-deficient diet-induced steatohepatitis [184]. Moreover, *S. boulardii* improved liver fibrosis, inflammation, injury (per transaminase levels), lipid peroxidation, intestinal permeability and plasma lipopolysaccharide levels in an experimental model of carbon tetrachloride-induced liver fibrosis in rats [185]. Even in an obstructive jaundice model (bile duct ligation) in rats, *S. boulardii* was found to decrease bacterial translocation into blood, mesenteric lymph nodes, liver and spleen, although it did not improve biochemical cholestasis and liver injury markers [186]. However, in a small trial involving 18 patients with cirrhosis, oral supplementation with 1×10^9 cells of *S. boulardii* three times daily over 30 days did not ameliorate intestinal permeability [187]. Administration of *S. cerevisiae* attenuates AFB1 liver toxicity in piglets, leading to significantly lower liver transaminases and hepatic AFB1 concentrations, and preventing histological features of aflatoxicosis [188]. Nevertheless, one must also consider the risks of fungal probiotic administration, since it can be associated with significant mortality and morbidity, in particular in immunosuppressed or critically ill patients, as indicated by a case series of iatrogenic *S. cerevisiae* fungemia [189]. Although fungal probiotic administration has been shown to be beneficial in multiple clinical trials in intestinal diseases, such as irritable bowel syndrome [190] or inflammatory bowel disease [191, 192], clinical evidence in hepatobiliary disease is largely lacking despite promising pre-clinical data. More human trials are hence warranted to evaluate the value of fungal probiotics in hepatobiliary conditions.

Conclusions

Mycobiome changes have now been established in essentially all hepatobiliary conditions. However, they are still a kind of dark matter, as we often do not know their true identity and characteristics, since we cannot culture many of them. Moreover, current methods often have some bias toward the most prevalent fungal populations or certain subpopulations, which might impede direct comparisons of studies that rely on different methodologies. Further, they do not colonise mice easily, so we do not have good mouse models. Nevertheless, we know that some fungi are hepatotoxic themselves and not just bystanders – for example, rodents colonised with *C. albicans* develop liver disease without additional stimuli. Fungi might hence possibly exacerbate liver disease in a two-hit model, one hit being alcohol, western diet, or a toxin, and another hit being the presence and deleterious impact of fungi. We are learning more and more about the mechanisms by which fungi contribute to liver disease, be it via fungal cell wall components or secreted toxins, such as beta-glucans or candidalysin, or fungal metabolites including prostaglandins. This knowledge will help us to develop innovative and personalised therapies to better treat these diseases in the future.

Financial support

This work was supported in part by National Institutes of Health (NIH) grant K12 HD85036, University of California San Diego Altman Clinical and Translational Research Institute (ACTRI)/NIH grant KL2TR001444, Pinnacle Research Award in Liver Diseases Grant #PNC22-159963 from the American Association for the Study of Liver Diseases Foundation (to P.H.), NIH grants R01 AA24726, R37 AA020703, U01 AA026939, U01 AA026939-04S1, by Award Number BX004594 from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development, and a Harrington Discovery Institute Foundation Grant (to B.S.) and services provided by NIH centers P30 DK120515 and P50 AA011999.

Conflict of interest

B.S. has been consulting for Ambys Medicines, Ferring Research Institute, Gelesis, HOST Therabiomics, Intercept Pharmaceuticals, Mabwell Therapeutics, Patara Pharmaceuticals and Takeda. B.S.'s institution UC San Diego has received grant support from Artizan Biosciences, Axial Biotherapeutics, BiomX, CymaBay Therapeutics, NGM Biopharmaceuticals, Prodigy Biotech and Synlogic Operating Company. B.S. is founder of Nterica Bio. UC San Diego has filed several patents with B.S. as inventor related to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Abbreviations

AFB1	aflatoxin B1
AH	alcohol-associated hepatitis
ALD	alcohol-associated liver disease
ALT	alanine aminotransferase
ASCA	anti-*Saccharomyces cerevisiae* antibodies
AUD	alcohol use disorder
C.	*Candida*
ECE1	extent of cell elongation 1

HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
IL-	interleukin-
ITS	internal transcribed spacer
MELD	model for end-stage liver disease
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NLRP3/6	nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3/6
OR	odds ratio
PBC	primary biliary cholangitis
PGE2	prostaglandin E2
PSC	primary sclerosing cholangitis
qPCR	quantitative PCR
S.	*Saccharomyces*
T2DM	type 2 diabetes mellitus
Th	T helper
TLRs	Toll-like receptors

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Key points:

- Despite a relatively small number of fungal cells in the human body, fungi are involved in the development of liver and biliary diseases.
- Various rodent models of fungal microbiome (mycobiome) modulation, including increasing the fungal burden (*e.g.* colonisation with fungi) or decreasing the fungal burden (*i.e.* via antifungals), have demonstrated the impact of the mycobiome on hepatobiliary conditions.
- Fungal products including toxins and metabolites can exacerbate liver and biliary diseases.
- In particular, the genus *Candida* and the species *Candida albicans* play a central role in the pathogenesis and progression of essentially all hepatobiliary conditions.
- Serum antibodies against fungal populations have predictive value with regard to disease severity and survival in hepatobiliary diseases.
- Development of mycobiome-based therapeutics will allow for innovative and personalised approaches to better treat these diseases.

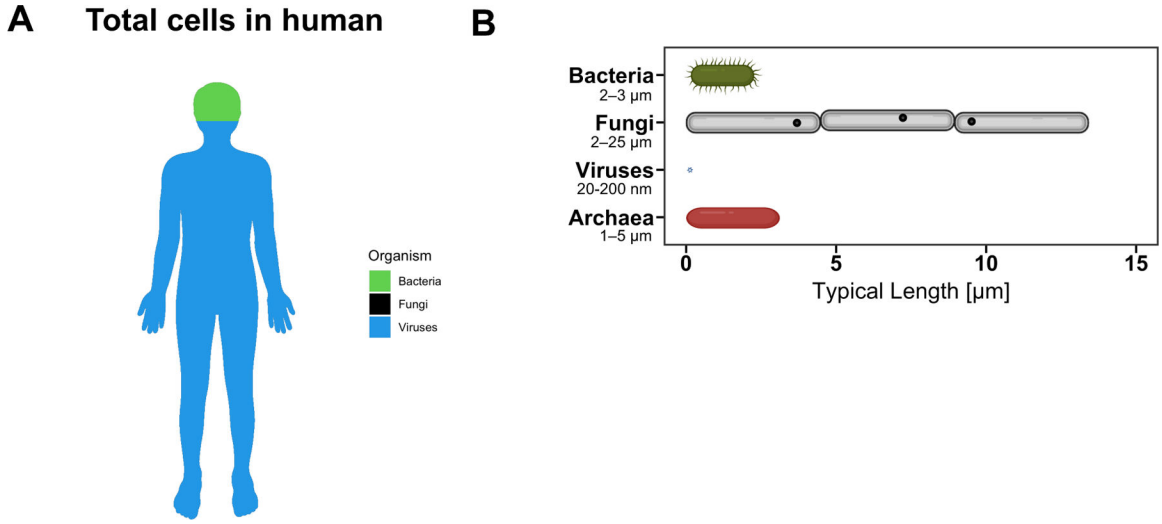

Fig. 1. The fungal microbiome in numbers and how it compares to other microbiome populations.

(A) Estimated total microbes in and on the human body. There are 10-fold more viruses (380×10^{12}) than bacteria (38×10^{12}), which are themselves likely ~200,000-fold more numerous than fungi ($\sim2\times10^{8}$, based on fungal stool estimates [9–11]). Due to this very small number compared with viruses (blue) and bacteria (green), the fungal subpopulation (black) is hence not visible in this figure. (B) Typical lengths of bacteria, fungi, viruses, and archaea. Fungi are typically larger (~10 times longer, 2–25 µm) than bacteria (2–3 µm) and archaea (1–5 µm), which are much larger than viruses (20–200 nm). Created with R statistical software, R version 2022.02.3 for Mac, 2022, the R Foundation for Statistical Computing, and with a license from Biorender.com.

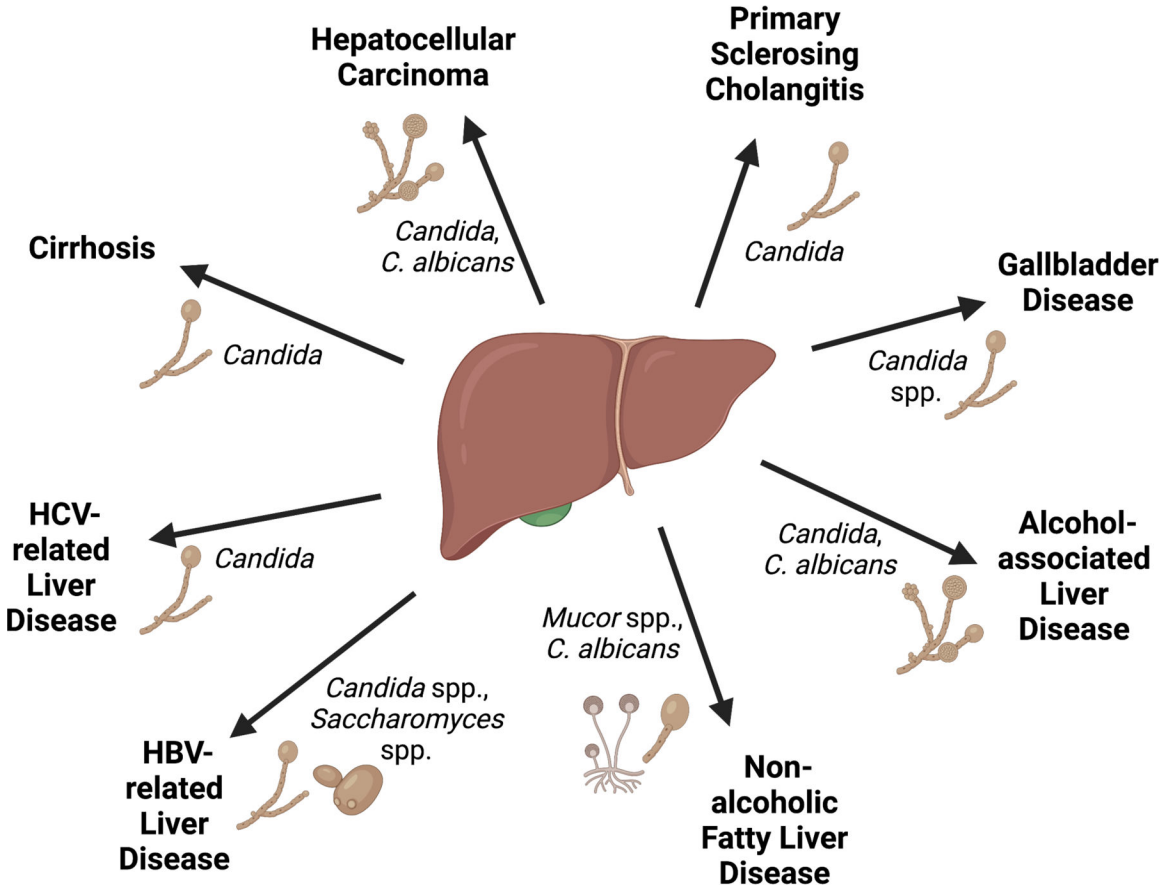

Fig. 2. Common fungal microbiome changes in hepatobiliary diseases.

Increases in the abundance of the genus *Candida* and its species *Candida albicans* have been associated essentially with all liver and biliary diseases. HBV, hepatitis B virus; HCV, hepatitis C virus. Created with a license from Biorender.com.

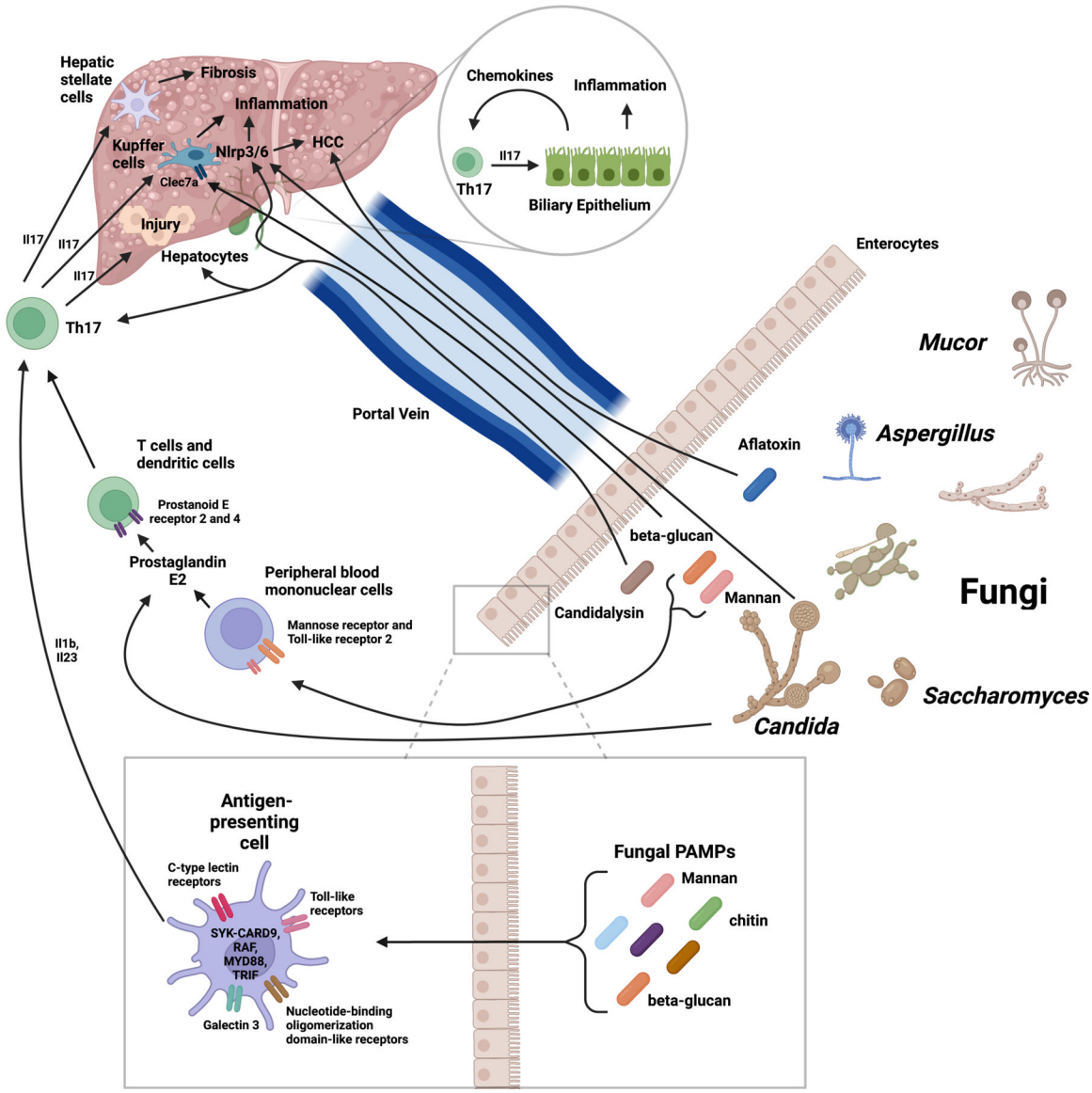

Fig. 3. Mechanisms by which fungi contribute to hepatobiliary diseases.

Fungal PAMPs such as beta-glucans and mannans induce antigen-presenting cells via various receptors, including C-type lectin receptors, Toll-like receptors, NLRs, or galectin 3. This triggers signalling cascades (such as SYK-CARD9, RAF, MYD99, TRIF pathways) to produce cytokines, *e.g.* IL-1β, IL-6, and IL-23, which activate IL-17A-producing Th17 cells. Fungal PAMPs also induce mononuclear cells to produce prostaglandin E2, which various fungi including *Candida albicans* can produce themselves as well. Prostaglandin E2 activates Th17 cells, which promote a fibrogenic, inflammatory, and cell death response by hepatic stellate cells, Kupffer cells, and hepatocytes, respectively. It also induces cholangiocytes to mount an inflammatory response and to produce chemokines and cytokines to maintain and mature Th17 cells. The toxin candidalysin, secreted by *Candida*, activates Th17 cells, damages hepatocytes, and amplifies the NLRP3 inflammasome promoting inflammation. Beta-glucans also induce inflammation via C-type lectin-like receptor Clec7a (=Dectin-1) on

Kupffer cells and macrophages. *Candida* activates NLRP6, exacerbating the development of HCC. Aflatoxins such as aflatoxin B1, secreted by *Aspergillus* spp., cause HCC by inducing DNA strand breaks, oxidative stress, adduct formation, and gene mutations. Created with a license from Biorender.com. HCC, hepatocellular carcinoma; NLRs, nucleotide-binding oligomerization domain-like receptors; NLRP3/6, NLR family pyrin domain-containing 3/6; PAMPs, pathogen-associated molecular patterns; Th, T helper.

Table 1.

Mycobiome changes in liver and biliary diseases.

References	Study participants	Biospecimen	Method	Genus	Species
Primary sclerosing cholangitis					
Lemoinne *et al.*, 2020	Primary sclerosing cholangitis (n = 22) *vs.* controls (n = 30)	Stool	ITS2	↑ *Exophiala*	↓ *Saccharomyces cerevisiae*
Rühlemann *et al.*, 2020	Primary sclerosing cholangitis (n = 33) *vs.* controls (n = 66)	Stool	ITS4	↑ *Candida*	↑ *Humicola griseum*
				↑ *Humicola*	
Xu *et al.*, 2022	Post cholecystectomy (n = 52) *vs.* controls (n = 52)	Stool	ITS1		↑ *Candida glabrata*
					↑ *Aspergillus* unassigned
					↓ *Candida albicans*
Alcohol-associated liver disease					
Lang *et al.*, 2020	Alcohol-associated hepatitis (n = 59), alcohol use disorder (n = 15) *vs.* controls (n = 11)	Stool	ITS1	↑ *Candida*	
				↓ *Penicilllium*	
				↓ *Saccharomyces*	
				↓ *Debaryomyces*	
Yang *et al.*, 2017	Alcohol-associated cirrhosis (n = 4), alcohol-associated hepatitis (n = 6), alcohol use disorder (n = 10) *vs.* controls (n = 8)	Stool	ITS1	↑ *Candida*	
				↓ *Epicoccum*	
				↓ *Debaryomyces*	
Chu *et al.*, 2020	Alcohol-associated hepatitis (n = 91), alcohol use disorder (n = 42) *vs.* controls (n = 11)	Stool	qPCR, culture		↑ *Candida albicans*
Hartmann *et al.*, 2021	Alcohol use disorder (n = 66) *vs.* controls (n = 18)	Stool	ITS2	↑ *Candida*	↑ *Candida albicans*
				↑ *Debaryomyces*	↑ *Candida zeylanoides*
				↑ *Pichia*	↑ *Issatchenkia orientalis*
				↑ *Kluyveromyces*	↑ *Scopulariopsis cordiae*
				↑ *Issatchenkia*	↓ *Kazachstania humilis*
				↑ *Scopulariopsis*	
				↓ *Aspergillus*	
	Active alcohol use disorder *vs.* abstinent alcohol use disorder (n = 56, paired)			↑ *Candida*	↑ *Candida albicans*
				↑ *Malassezia*	↑ *Candida zeylanoides*

References	Study participants	Biospecimen	Method	Genus	Species
				↑ *Hanseniaspora*	↑ *Malassezia restricta*
				↑ *Kluyveromyces*	↑ *Cyberlindneria jadinii*
				↑ *Cyberlindneria*	↑ *Issatchenkia orientalis*
				↑ *Pichia*	
				↑ *Issatchenkia*	
				↑ *Claviceps*	
				↓ *Trichosporon*	
Non-alcoholic fatty liver disease					
Demir *et al.*, 2022	Non-alcoholic steatohepatitis (n = 54) *vs.* non-alcoholic fatty liver (n = 24)	Stool	ITS2		↑ *Candida albicans*
					↑ *Pichia barkeri*
					↑ *Mucor* species
					↑ *Cyberlindneria jadinii*
					↓ *Malassezia* species
	Non-alcoholic steatohepatitis (n = 54) *vs.* control (n = 16)				↑ log ratio *Babjeviella inositovora*/ *Saccharomyces cerevisiae*
					↑ log ratio *Mucor* species/ *Saccharomyces cerevisiae*
	Fibrosis stages F2-F4 (n = 38) *vs.* F0-F1 (n = 40)				↑ *Candida albicans*
					↑ *Pichia barkeri*
					↑ *Mucor* species
					↑ *Cyberlindneria jadinii*
					↓ *Penicillium* species
					↓ *Blumeria* species
	Fibrosis stages F2-F4 (n = 38) *vs.* control (n = 16)				↑ log ratio *Babjeviella inositovora*/ *Saccharomyces cerevisiae*
					↑ log ratio *Mucor* species/ *Saccharomyces cerevisiae*
You *et al.*, 2021					
	Non-alcoholic fatty liver disease (n = 79) *vs.* control (n = 34)	Stool	ITS2	↑ *Talaromyces*	
				↑ *Paraphaeosphaeria*	
				↑ *Lycoperdon*	
				↑ *Curvularia*	
				↑ *Phialemoniopsis*	

References	Study participants	Biospecimen	Method	Genus	Species
				↑ *Paraboeremia*	
				↑ *Sarcinomyces*	
				↑ *Cladophialophora*	
				↑ *Sordaria*	
				↓ *Leptosphaeria*	
				↓ *Pseudopithomyces*	
				↓ *Fusicolla*	
	Non-alcoholic steatohepatitis (n = 15) *vs.* non-alcoholic fatty liver (n = 17)			↑ *Paramycosphaerella*	
				↑ *Fusicolla*	
				↑ *Arthrinium*	
				↑ *Triparticalcar*	
				↑ *Trichoderma*	
				↑ *Cladosporium*	
	Fibrosis stages F2-F4 (n = 10) *vs.* F0-F1 (n = 22)			↑ *Cladosporium*	
				↑ *Staphylotrichum*	
				↑ *Paecilomyces*	
				↑ *Thermomyces*	
				↓ *Pulvinula*	
Non-alcoholic fatty liver disease *vs.* alcohol-associated liver disease					
Demir *et al.*, 2022	Non-alcoholic fatty liver disease with fibrosis stages F3-F4 (n = 24) *vs.* alcohol use disorder with F3-F4 (n = 11)	Stool	ITS2		↑ *Mucor* species
					↓ *Candida albicans*
					↓ *Candida* species
					↓ *Debaryomyces* species
					↓ *Blumeria* species
Hepatitis B virus					
Guo *et al.*, 2010	Hepatitis B virus-cirrhosis (n = 80), chronic hepatitis B (n = 68), hepatitis B virus carriers (n = 66) *vs.* controls (n = 84)	Stool	qPCR		↑ *Candida albicans*
					↑ *Candida parapsilosis*
					↑ *Candida glabrata*
					↑ *Candida krusei*
					↑ *Candida tropicalis*
					↑ *Saccharomyces cerevisiae*
Chen *et al.*, 2011	Hepatitis B virus-cirrhosis (n = 38), chronic hepatitis B (n = 35), hepatitis B virus carriers (n = 33) *vs.* controls (n = 55)	Stool	qPCR, culture		↑ *Aspergillus versicolor*
					↑ *Aspergillus penicillioides*
					↑ *Candida solani*
					↑ *Candida albicans*

References	Study participants	Biospecimen	Method	Genus	Species
					↑ *Candida austromarina*
					↑ *Candida intermedia*
					↑ *Candida milleri*
					↑ *Candida tropicalis*
					↑ *Saccharomyces* species
					↑ *Saccharomyces cerevisiae*
					↑ *Galactomyces geotrichum*
					↑ *Simplicillium obclavatum*
					↑ *Simplicillium lanosoniveum*
					↑ *Chaetomium* species
					↑ *Rhizopus microsporus* var.
					↑ *Wallemia muriae*
					↓ *Penicillum freii*
					↓ *Malassezia pachydermatis*
Mou *et al.*, 2018	Hepatitis B virus-cirrhosis (n = 52), chronic hepatitis B (n = 52) *vs.* controls (n = 40)	Stool	Culture	↑ *Saccharomyces*	
Hepatitis C virus					
Mashaly *et al.*, 2017	Hepatitis C virus-cirrhosis (n = 26), chronic hepatitis C (n = 27) *vs.* controls (n = 55)	Stool	qPCR, culture	↑ *Candida*	
Cirrhosis					
Bajaj *et al.*, 2018	Cirrhosis inpatient (n = 66; 16 hepatitis C virus, 17 alcohol, 15 hepatitis C virus+alcohol, 11 non-alcoholic fatty liver disease, 7 others) *vs.* cirrhosis outpatient (n = 77; 33 hepatitis C virus, 9 alcohol, 8 hepatitis C virus+alcohol, 18 non-alcoholic fatty liver disease, 9 others) *vs.* controls (n = 26)	Stool	ITS1	↑ *Candida*	
Hepatocellular carcinoma					
Liu *et al.*, 2022	Hepatocellular carcinoma (n = 17) *vs.* cirrhosis (n = 11)	Stool	ITS1	↑ *Candida*	↑ *Candida albicans*
				↓ *Kazachstania*	
				↓ *Debaryomyces*	
				↓ *Xeromyces*	
				↓ *Amorphotheca*	
				↓ *Blastobotrys*	

ITS, internal transcribed spacer; qPCR, quantitative PCR.