Old Herborn University Seminar Monograph 35: Microbes impacting mammalian systems biology.
Editors: Peter J. Heidt, Tore Midtvedt, Andreas Schwiertz, James Versalovic, and Volker Rusch.
Old Herborn University Foundation, Herborn, Germany: 25-35 (2023).

TRIPARTITE INTERACTIONS AMONG FUNGI, BACTERIA AND THE MAMMALIAN HOST

MARILENA PARIANO[1], MATTEO PUCCETTI[2], GIORGIA RENGA[1], and LUIGINA ROMANI[1]

[1]Department of Medicine and Surgery, and [2]Department of Pharmaceutical Science, University of Perugia, Perugia, 06132, Italy

SUMMARY

Fungal infections are difficult diseases to manage in humans. Developing antifungal drugs is an exceptionally challenging task due to the close evolutionary relationship between fungi and humans, making it hard to find fungal-specific inhibitors without toxicity to humans. Optimal implementation of antifungal treatments will require improved understanding of not only antifungal resistance mechanisms, but also of the tripartite interactions between fungi, bacteria and the mammalian host. We emphasized in this review how the definition of the mechanisms behind this tripartite interaction may provide us with an understanding of multi-kingdom community processes that allows for the development of novel therapeutic approaches for human fungal diseases.

INTRODUCTION

Some fungi cause diseases in healthy people, but most fungal infections occur in individuals already experiencing serious illness, such as cancer, solid organ and hematopoietic stem cell transplantation, intensive care and recently COVID-19 (*Brown* et al., 2012). Among the estimated 1.5–5.0 million fungal species on planet Earth (*O'Brien* et al., 2005) only several hundred cause disease in humans, and very few are able to affect healthy people. Animals co-evolved with fungi, and the sophisticated and potent human immune system arose from the constant challenge posed by the microbial world. Fungal pathogens likely adapted their pathogenic repertoire to other animal prey - mammals, insects, and even unicellular amoebae - before encountering and attacking humans (*Casadevall*, 2012). For a fungus, parasitizing a human is a demanding strategy that demands, among others, to withstand the human immune system. Although not unique among infectious agents, fungi possess complex and unusual relationships with the vertebrate immune system, partly due to some prominent features (see below).

Developing antifungal drugs is an exceptionally challenging task due to the close evolutionary relationship between fungi and humans, making it hard to find fungal-specific inhibitors without toxicity to humans. Only a few antifungal drugs have been approved in the past few decades (*McCarty and Pappas*, 2021). Although these drugs are effectively used for current treatments, there are some drawbacks to prolonged usage, including the emergence of multi-drug resistant species of *Candida auris* (*Forsberg* et al., 2019) and the increased incidence of voriconazole-resistant *Aspergillus fumigatus*

isolates (*van der Linden* et al., 2013). This underscores the need for new approaches in the management of fungal diseases. A renewed focus on repurposing of drugs used for other diseases provides a promising approach for antifungal drug discovery because their pharmacodynamic, pharmacokinetic, and toxicity profiles are well established (*Bouz and Dolezal*, 2021). In addition, alternative therapies, such as immune cell therapies, vaccines, and monoclonal antibodies, have shown potential in animal models, although these are not yet available for clinical use (*Bernardes and Hohl*, 2020). Optimal implementation of antifungal treatments will require improved understanding of not only target and drug efflux-based antifungal resistance mechanisms, but of the tripartite interactions between fungi, bacteria and the mammalian host. Despite the abundance of bacterial–fungal interactions in nature and the clinical environment, very little is known about the molecular mechanisms underlying these interactions and their importance to human health. Unravelling the mechanisms that microorganisms use in a competitive, polymicrobial environment would not only deepen our understanding of microbial pathogenesis but may also provide important insights into novel pathways that are amenable for the development of new antimicrobial drugs. History has demonstrated the power of understanding such interactions, with the identification of penicillin being the consequence of a bacterial–fungal interactions on a contaminated agar plate.

BACTERIAL-FUNGAL INTERACTIONS IN THE GASTROINTESTINAL TRACT

Fungi and bacteria coexist creating complex communities that are important in agriculture and human health. Bacteria comprise more than 90% of the human microbiome, most belonging to four phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria (*Qin* et al., 2010). The human microbiome also harbours other microbes such as fungi, accounting for ~ 0.1% of the human gut microbiome by shotgun sequencing, viruses (bacteriophages), archaea, and protozoans, collectively making a median 1.5×10^{11} cell counts per gram of faecal material. The study of the gut mycobiota from healthy stool identified Ascomycota and Basidiomycota as the most abundant taxa, and *Saccharomyces, Candida, Malassezia* and *Cladosporium* as the dominant genera (*Nash* et al., 2017). Of interest, the most abundant genera of fungi in the guts of mice were also those present in humans (*Dollive* et al., 2013). A number of studies have indicated that the gut mycobiome (*Wang* et al., 2023) and its interactions with bacterial species (*Santus* et al., 2021) influence gut homeostasis and are relevant to human health. Indeed, beneficial interactions between bacteria and fungi are continuously being explored as potential probiotic interventions for intestinal disease. *Candida albicans* is a member of the intestinal microbiota in the majority of the human population. This underscores *C. albicans'* adaptation to life in the intestine without inducing competitive interactions with other microbes or immune responses detrimental to its survival. Specific conditions such as a dysbalanced microbiome, a suppression of the immune system, and an impaired intestinal barrier can predispose for invasive, mostly nosocomial *C. albicans* infections. Colonization of the intestine and translocation through the intestinal barrier are fundamental

aspects of the processes preceding life-threatening systemic candidiasis. However, protective effects have also been described, including the ability of this commensal to orchestrate the usage of multiple receptor-signalling pathways in dendritic cells, ultimately affecting antifungal resistance and tolerance (see below) (*Romani* et al., 2002; *Romani*, 2011). Interestingly, *C. albicans* also demonstrates probiotic properties by enhancing the growth of two strictly anaerobic commensal bacteria, *Bacteroides fragilis* and *Bacteroides vulgatus*, likely via aerobic respiration and/or antioxidant production (*Valentine* et al., 2019). As a matter of fact, the administration of antifungal drugs exacerbated colitis (*Wheeler* et al., 2016) while *C. albicans* affects the recolonization of the cecum by the microbiota in mice treated with antibiotics (*Mason* et al., 2012; *Erb Downward* et al., 2013). Although antibiotic-treated *C. albicans*-colonized mice showed reduced expression of specific immune response genes it is also likely that fungal yeasts directly interact with bacteria. The yeast *Saccharomyces boulardii* has indeed been extensively studied as a potential probiotic due to its protective effect against various bacterial gastrointestinal pathogens, including *Clostridiodes difficile, Helicobacter pylori, Vibrio cholerae, Salmonella enterica serovar Typhimurium, Shigella flexneri, and Escherichia coli (Ansari* et al., *2023)*.

Fungus-bacterium interactions are however bidirectional with a variety of reciprocal interactions encompassing antagonistic and pathogenetic interactions in addition to beneficial ones (*Nogueira* et al., 2019; *Santus* et al., 2021; *Zhang* et al., 2022). For instance, the abundance of fungi is regulated by gut bacteria. Firmicutes and Bacteroidetes restrict colonization of *C. albicans* in the mouse gut by activating transcription factor HIF-1α in intestinal cells, which causes an increase in the antimicrobial peptide LL-37 (*Fan* et al., 2015). *C. albicans* and lactic acid bacteria (LAB) have the same metabolic niches throughout the gastrointestinal tract. Dysbiosis of *C. albicans* causes altered levels of LAB, especially *Lactobacillus* spp. and *Enterococcus* spp. Lactobacilli inhibit *C. albicans*, while *Enterococcus faecalis* and *C. albicans* are mutualistic (*Zeise* et al., 2021). In an in vitro gut model, *L. rhamnosus* modified the metabolic environment, altering the expression of virulence-related genes and reducing *C. albicans* induced epithelial damage (*Graf* et al., 2019; *Alonso-Roman* et al., 2022). Moreover, probiotics strains, such as *L. acidophilus*, *L. reuteri, L. casei GG*, and *Bifidobacterium* spp., have shown efficacy in limiting the severity of *C. albicans* infections in both immunocompromised and germ-free mice (*Wagner* et al., 1997) and inhibiting the in vitro formation of polymicrobial biofilms (*Hager* et al., 2019). Furthermore, metabolites produced by a consortium of bacterial species derived from healthy human faecal samples effectively inhibited the growth of *C. albicans* in liquid culture. Species of *Roseburia* and *Bacteroides ovatus* were directly responsible for these antifungal effects (*Garcia* et al., 2017).

Alternatively, interactions between fungi and bacteria have the potential to enhance microbial pathogenesis. For instance, both *C. albicans* and *S. cerevisiae* enhance the pathogenicity of the opportunistic pathogen *Acinetobacter baumannii* by producing ethanol (*Smith* et al., 2004) or the quorum-sensing molecule farnesol (*Peleg* et al., 2008). *C. albicans* allows the growth of strict anaerobes, including *C. difficile*, both in vivo (*Panpetch* et al., 2019) and in vitro (*van Leeuwen* et al., 2016), under aerobic conditions due to the rapid reduction of dissolved oxygen in the vicinity of

the yeast (*Lambooij* et al., 2017) and *C. difficile* inhibited *C. albicans* hyphal growth through the secretion of the small molecule p-cresol (*van Leeuwen* et al., 2016). However, another study found that prior colonization of mice with *C. albicans* protected mice from *C. difficile* infection (*Markey* et al., 2018), a finding implicating the effects of *Candida* colonization on mucosal immune homeostasis. Finally, while Enterobacteriaceae were required for *C. albicans*-mediated enhancement of colitis (*Sovran* et al., 2018), likely due to the ability of enterohemorrhagic *E. coli* to enhance *C. albicans* epithelial invasion vitro (*Yang* et al., 2016), *S. marcescens* employed a type VI secretion system to deliver antifungal toxins to kill *C. albicans* (*Trunk* et al., 2018) and S. *typhimurium* employed a type III secretion system to block hyphal formation (*Kim and Mylonakis*, 2011). Thus, these few examples highlight the complex nature of fungal-bacterial interactions in the mammalian gut, and the broad impact of fungi on bacterial species within microbiomes (*Pierce* et al., 2021). The spectrum of findings points to new possibilities and challenges in addressing the global spread of drug-resistant fungal pathogens and the diminishing pipelines of effective antifungal drugs (*Chow* et al., 2023). Ultimately, the crucial role of the fungal-bacterial interactions across habitats and ecosystems is well established (*Deveau* et al., 2018; *Steffan* et al., 2020).

TRIPARTITE INTERACTIONS: FUNGI, BACTERIA AND THE MAMMALIAN HOST

Like other microorganisms, fungi interact with the immune system at mucosal surfaces in ways that are important both for host defence and for regulating the immune system (*Underhill and Iliev*, 2014). As said, fungi possess complex and unusual relationships with the vertebrate immune system, including their ability to exist in different forms and to reversibly switch from one to the other in infection. Examples are the dimorphic fungi (*H. capsulatum, P. brasiliensis, C. immitis, and B. dermatitidis*) which transform from saprobic filamentous molds to unicellular yeasts in the host, the filamentous fungi (such as *Aspergillus* spp.) that, inhaled as unicellular conidia, may transform into a multicellular mycelium, and some species of *Candida*, capable of growing in different forms such as yeasts, blastospores, pseudohyphae and hyphae. This implicates the existence of a multitude of recognition and effector mechanisms to oppose fungal infectivity at the different body sites. For commensals, two prominent features are important. The highly effective strategies of immune evasion have evolved to enable survival in the host environment and the prolonged antigenic stimulation of the host can have profound immunoregulatory consequences. Thus, in the context of the antagonistic relationships that characterize the host-pathogen interactions, the strategies used by the host to limit fungal infectivity are necessarily disparate and, in retaliation, fungi have developed their own elaborate tactics to evade or overcome these defences (*Romani*, 2004, 2011). This may have resulted in an expanded repertoire of cross-regulatory and overlapping antifungal host responses. Indeed, through the involvement of different pattern recognition receptors, cells of the innate immune system not only discriminate between the different forms of fungi, but also contribute to resistance and tolerance to fungi at the level of the adaptive T helper immunity (*Romani*, 2004, 2011; *Underhill and Iliev*, 2014). Resistance is

the ability of the host to reduce the success of infection or to increase the rate of clearance of the pathogens. Tolerance is the ability to reduce the detrimental effects of the pathogens on the performances of the hosts, either directly or by limiting immunopathological mechanisms. Infectivity diminishes naturally among resistant hosts but not necessarily among tolerant ones, as these harbour the pathogen with no or moderate loss in performance. Resistance and tolerance are associated with fitness costs, which arise from the diversion of limiting resources away from biological processes related to performance. The host organism is a complex mosaic of cell populations that requires adequate supplies of nutrients for maintenance, growth and proliferation. Because many nutrient requirements may be shared by host cells, pathogens and indigenous microbiota, all these cells may potentially compete for growth-limiting resources.

THE SHARING OF TRYPTOPHAN METABOLISM

Amino acid metabolic pathways are crucial regulators of immunity from plants (*Zeier*, 2013) to mammals (*Grohmann and Bronte*, 2010). Amino acids serve as the building blocks of proteins, so their importance in immune activity requiring cell division, cytokine and chemokine production and other de novo protein synthesis requirements is self-evident. In addition to this process, some amino acids, or their downstream metabolites, have been implicated as anti-microbial agents. Tryptophan (Trp) is a central hub for host/microbial information processing. Trp is an essential amino acid for humans and must be obtained from the diet. Besides being involved in protein synthesis, Trp is a versatile precursor and can be metabolized by both host and microbial enzymes to generate a variety of molecules involved in different fundamental processes (*Borghi et al.,* 2020; *Grifka-Walk et al.,* 2021; *Li et al.,* 2022; *Seo and Kwon*, 2023). Three pathways have gained considerable interest for their role at the interface between the host, the microbiome and pathogens. These pathways include the host kynurenine and serotonin pathways and the microbial indole pathway (*Seo and Kwon*, 2023). The host kynurenine pathway and the microbial indole pathway, converge on a central xenobiotic receptor, the Aryl Hydrocarbon Receptor (AhR), a critical regulator at the host/microbe interface (*Zelante et al.,* 2013; *Metidji et al.,* 2018; *Dong and Perdew*, 2020; *Stockinger et al.,* 2021). The kynurenine pathway accounts for ~95% of overall Trp degradation and the first and rate-limiting step is mediated by indoleamine 2, 3–dioxygenase (IDO)1, along with IDO2 (a paralogue of IDO1) and the tryptophan 2,3-dioxygenase, TDO2, resulting in the formation of kynurenine. The kynurenine pathway, and IDO1 in particular, has been associated with the promotion of tolerance in different inflammatory conditions (*Cervenka et al.,* 2017). For instance, the Trp metabolic pathway crucially provides immune homeostasis in fungal infections by taming heightened inflammatory responses and inducing immune and tissue tolerance, an activity to which the host, fungi and the microbiota cooperatively contributed (*Romani et al.,* 2015). These results provide proof-of concept demonstration of the druggability of host metabolic pathways for antifungal tolerance defences. The serotonin pathway also influences the interactions between host and microbes. For instance, commensal bacteria regulate the synthesis of serotonin by the host

(*Yano et al.,* 2015), and serotonin may modulate the composition of the gut microbiome (*Stasi et al.,* 2019). The third important pathway is represented by the indole pathway whereby different species of bacteria produce indole compounds via the major metabolic pathways involving the activity of: i) tryptophanase generating indole, ii) aromatic amino acid aminotransferase generating indole-3-acetaldehyde and indole-3-aldehyde (3-IAld) and iii) tryptophan deaminase generating indole-3-pyruvic acid (*Morgan et al.,* 2023). Indoles are very attractive molecules as they have been shown to augment health span across a broad range of evolutionarily diverse species from different phyla, to control bacterial fitness, including antibiotic resistance and strengthening of the epithelial barrier function (*Wikoff et al.,* 2009; *Venkatesh et al.,* 2014; *Lee et al.,* 2015; *Dodd et al.,* 2017; *Roager and Licht* 2018; *Hendrikx and Schnabl* 2019; *Ornelas et al.,* 2022). Microbiota-derived indoles are ligands of AhR, thus suggesting that the host AhR has evolved to sense and respond to the presence of the microbiota resulting in maintenance of homeostasis. In addition to its function as xenobiotic receptor, AhR has been implicated in a wide range of physiological activities, including the bidirectional communication with the microbiome for modulating host immunity, tolerance and metabolism (*Zelante et al.,* 2013; *Metidji et al.,* 2018; *Stockinger et al.,* 2021; *Nieves et al.,* 2022).

Our group has previously identified a microbial pathway of Trp utilization that regulates *Candida* commensalism and mucosal homeostasis in the gut (*Zelante et al.,* 2013; *Roager and Licht* 2018; *Puccetti et al.,* 2023). Indeed, the commensal *L. reuteri*, by switching to Trp as energy source, produces 3-IAld via the aromatic amino acid aminotransferase pathway. 3-IAld, in turn, by working as a ligand of AhR, stimulated innate lymphoid cells to release IL-22 that efficiently controlled *C. albicans* colonization by promoting epithelial integrity and the release of antimicrobial peptides. The scenario emerging from these findings appear to trace the dichotomy of resistance versus tolerance back to the different pathways of Trp utilization. Indeed, the microbial AhR-IL-22 axis appear to promote resistance by means of primitive antifungal defence mechanisms that include the homeostatic maintenance of the triad microbiota, epithelium and immune system. In contrast, the host IDO1 pathway emerges as a functional specialization of antifungal tolerance mechanisms that evolved to facilitate the establishment of a fungal microbiome. It should also be noted that although the IDO1 product kynurenine and the microbial product 3-IAld are both ligands of AhR, the outcome may be different because AhR activation results in a variety of effects that depend, among others, on the ligand itself. Therefore, the IDO1 pathway and the microbial Trp metabolic pathway, although intersecting at a common node, may underlie distinct functional activities in the resistance vs tolerance antifungal response.

CONCLUSIONS

Fungi serve as a biological scaffold for bacterial attachment. Consequences of these interactions are not just limited to the respective microorganisms, but also have major impacts in the immune system resulting in a network of bidirectional interactions that operates across the health and disease spectrum (Figure 1). It is clear that individual microbes have important effects on the host, and

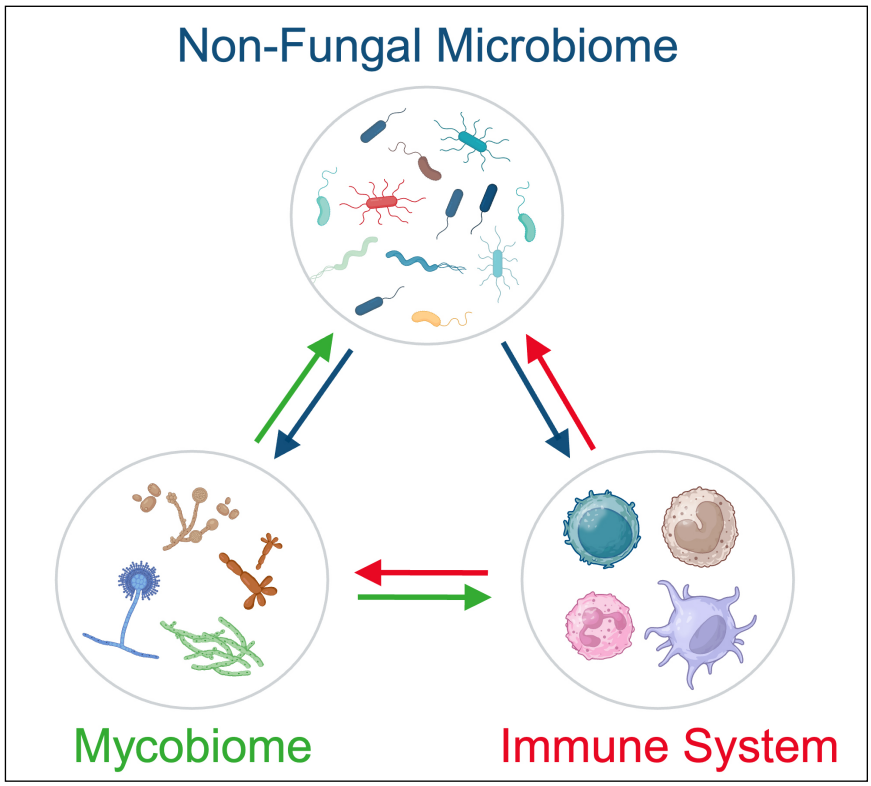

Figure 1: Schematic representation of the bidirectional network among the microbiome, the mycobiome and the immune system. The figure was made on BioRender.

that a balance of the microbiota is necessary for homeostasis. Examining the mechanisms behind the fungi/bacteria balance will provide us with an understanding of multi-kingdom community processes that allows for the development of disease-specific therapeutic approaches in different ecological settings. In so doing, we have discovered that harnessing of the AhR/IDO1 pathway may represent a much-needed strategy for improving and preventing the burden of human fungal diseases. By shifting the current view of pathogenesis from pathogen- to host-oriented views, we provided proof-of-concept evidence of the feasibility of therapeutic approaches to reduce infectious disease burden by targeting host and microbial-derived immunometabolic checkpoints leading to tolerance. The druggability of this pathway with microbial metabolites to promote homeostasis and microbial symbiosis at mucosal surfaces is becoming a reality.

ACKNOWLEDGEMENTS

This study was supported by the Specific Targeted Research Project MicroTher (ERC-2018-PoC-813099).

LITERATURE

Alonso-Roman, R., Last, A., Mirhakkak, M.H., Sprague, J.L., Moller, L., Grossmann, P., Graf, K., Gratz, R., Mogavero, S., Vylkova, S., Panagiotou, G., Schauble, S., Hube, B., and Gresnigt, M.S.: Lactobacillus rhamnosus colonisation antagonizes Candida albicans by forcing metabolic adaptations that compromise pathogenicity. Nat. Commun. 13, 3192 (2022).

Ansari, F., Alian Samakkhah, S., Bahadori, A., Jafari, S.M., Ziaee, M., Khodayari, M.T., and Pourjafar, H.: Health-promoting properties of Saccharomyces cerevisiae var. boulardii as a probiotic; characteristics, isolation, and applications in dairy products. Crit. Rev. Food Sci. Nutr. 63, 457-485 (2023).

Bernardes, M. and Hohl, T.M.: Fungal Infections Associated With the Use of Novel Immunotherapeutic Agents. Curr. Clin. Microbiol. Rep. 7, 142-149 (2020).

Borghi, M., Puccetti, M., Pariano, M., Renga, G., Stincardini, C., Ricci, M., Giovagnoli, S., Costantini, C., and Romani, L.: Tryptophan as a Central Hub for Host/Microbial Symbiosis. Int. J. Tryptophan Res. 13, 1178646920919755 (2020).

Bouz, G. and Dolezal, M.: Advances in Antifungal Drug Development: An Up-To-Date Mini Review. Pharmaceuticals (Basel) 14, 1312 (2021).

Brown, G.D., Denning, D.W., Gow, N.A., Levitz, S.M., Netea, M.G., and White, T.C.: Hidden killers: human fungal infections. Sci. Transl. Med. 4, 165rv13 (2012).

Casadevall, A.: Fungi and the rise of mammals. PLoS Pathog. 8, e1002808 (2012).

Cervenka, I., Agudelo, L.Z., and Ruas, J.L.: Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science 357, eaaf9794 (2017).

Chow, E.W.L., Pang, L.M., and Wang, Y.: Impact of the host microbiota on fungal infections: New possibilities for intervention? Adv. Drug Deliv. Rev. 198, 114896 (2023).

Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., Hacquard, S., Herve, V., Labbe, J., Lastovetsky, O.A., Mieszkin, S., Millet, L.J., Vajna, B., Junier, P., Bonfante, P., Krom, B.P., Olsson, S., van Elsas, J.D., and Wick, L.Y.: Bacterial-fungal interactions: ecology, mechanisms and challenges. FEMS Microbiol. Rev. 42, 335-352 (2018).

Dodd, D., Spitzer, M.H., Van Treuren, W., Merrill, B.D., Hryckowian, A.J., Higginbottom, S.K., Le, A., Cowan, T.M., Nolan, G.P., Fischbach, M.A., and Sonnenburg, J.L.: A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. Nature 551, 648-652 (2017).

Dollive, S., Chen, Y.Y., Grunberg, S., Bittinger, K., Hoffmann, C., Vandivier, L., Cuff, C., Lewis, J.D., Wu, G.D., and Bushman, F.D.: Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. PLoS One 8, e71806 (2013).

Dong, F. and Perdew, G.H.: The aryl hydrocarbon receptor as a mediator of host-microbiota interplay. Gut Microbes 12, 1859812 (2020).

Erb Downward, J.R., Falkowski, N.R., Mason, K.L., Muraglia, R., and Huffnagle, G.B.: Modulation of post-antibiotic bacterial community reassembly and host response by Candida albicans. Sci. Rep. 3, 2191 (2013).

Fan, D., Coughlin, L.A., Neubauer, M.M., Kim, J., Kim, M.S., Zhan, X., Simms-Waldrip, T.R., Xie, Y., Hooper, L.V., and Koh, A.Y.: Activation of HIF-1alpha and LL-37 by commensal bacteria inhibits Candida albicans colonization. Nat. Med. 21, 808-814 (2015).

Forsberg, K., Woodworth, K., Walters, M., Berkow, E.L., Jackson, B., Chiller, T., and Vallabhaneni, S.: Candida auris: The recent emergence of a multidrug-resistant fungal pathogen. Med. Mycol. 57, 1-12 (2019).

Garcia, C., Tebbji, F., Daigneault, M., Liu, N.N., Kohler, J.R.. Allen-Vercoe, E., and Sellam, A.: The Human Gut Microbial Metabolome Modulates Fungal Growth via the TOR Signaling Pathway. mSphere 2, e00555-17 (2017).

Graf, K., Last, A., Gratz, R., Allert, S., Linde,

S., Westermann, M., Groger, M., Mosig, A.S., Gresnigt, M.S., and Hube, B.: Keeping Candida commensal: how lactobacilli antagonize pathogenicity of Candida albicans in an in vitro gut model. Dis. Model. Mech. 12, dmm039719 (2019).

Grifka-Walk, H.M., Jenkins, B.R., and Kominsky, D.J.: Amino Acid Trp: The Far Out Impacts of Host and Commensal Tryptophan Metabolism. Front. Immunol. 12, 653208 (2021).

Grohmann, U. and Bronte, V.: Control of immune response by amino acid metabolism. Immunol. Rev. 236, 243-264 (2010).

Hager, C.L., Isham, N., Schrom, K.P., Chandra, J., McCormick, T., Miyagi, M., and Ghannoum, M.A.: Effects of a Novel Probiotic Combination on Pathogenic Bacterial-Fungal Polymicrobial Biofilms. mBio 10, e00338-19 (2019).

Hendrikx, T. and Schnabl, B.: Indoles: metabolites produced by intestinal bacteria capable of controlling liver disease manifestation. J. Intern. Med. 286, 32-40 (2019).

Kim, Y. and Mylonakis, E.: Killing of Candida albicans filaments by Salmonella enterica serovar Typhimurium is mediated by sopB effectors, parts of a type III secretion system. Eukaryot. Cell 10, 782-790 (2011).

Lambooij, J.M., Hoogenkamp, M.A., Brandt, B.W., Janus, M.M., and Krom, B.P.: Fungal mitochondrial oxygen consumption induces the growth of strict anaerobic bacteria. Fungal Genet. Biol. 109, 1-6 (2017).

Lee, J.H., Wood, T.K., and Lee, J.P.: Roles of indole as an interspecies and interkingdom signaling molecule. Trends Microbiol. 23, 707-718 (2015).

Li, Y., Liu, N., Ge, Y., Yang, Y., Ren, F., and Wu, Z.: Tryptophan and the innate intestinal immunity: Crosstalk between metabolites, host innate immune cells, and microbiota. Eur. J. Immunol. 52, 856-868(2022).

Markey, L., Shaban, L., Green, E.R., Lemon, K.P., Mecsas, J., and Kumamoto, C.A.: Precolonization with the commensal fungus Candida albicans reduces murine susceptibility to Clostridium difficile infection. Gut Microbes 9, 497-509 (2018).

Mason, K.L., Erb Downward, J.R., Mason, K.D., Falkowski, N.R., Eaton, K.A., Kao, J.Y., Young, V.B., and Huffnagle, G.B.: Candida albicans and bacterial microbiota interactions in the cecum during recolonization following broad-spectrum antibiotic therapy. Infect Immun 80, 3371-3380 (2012).

McCarty, T.P. and Pappas, P.G.: Antifungal Pipeline. Front. Cell. Infect. Microbiol. 11, 732223 (2021).

Metidji, A., Omenetti, S., Crotta, S., Li, Y., Nye, E., Ross, E., Li, V., Maradana, M.R., Schiering, C., and Stockinger, B.: The Environmental Sensor AHR Protects from Inflammatory Damage by Maintaining Intestinal Stem Cell Homeostasis and Barrier Integrity. Immunity 49, 353-362.e35 (2018).

Morgan, E.W., Dong, F., Annalora, A.J., Murray, I.A., Wolfe, T., Erickson, R., Gowda, K., Amin, S.G., Petersen, K.S., Kris-Etherton, P.M., Marcus, C.B., Walk, S.T., Patterson, A.D., and Perdew, G.H.: Contribution of Circulating Host and Microbial Tryptophan Metabolites Toward Ah Receptor Activation. Int. J. Tryptophan Res. 16, 11786469231182510 (2023).

Nash, A.K., Auchtung, T.A., Wong, M.C., Smith, D.P., Gesell, J.R., Ross, M.C., Stewart, C.J., Metcalf, G.A., Muzny, D.M., Gibbs, R.A., Ajami, N.J., and Petrosino, J.F.: The gut mycobiome of the Human Microbiome Project healthy cohort. Microbiome 5, 153 (2017).

Nieves, K.M., Hirota, S.A., and Flannigan, K.L.: Xenobiotic receptors and the regulation of intestinal homeostasis: harnessing the chemical output of the intestinal microbiota. Am. J. Physiol._Gastrointest. Liver Physiol. 322, G268-G281 (2022).

Nogueira, F., Sharghi, S., Kuchler, K., and Lion, T.: Pathogenetic Impact of Bacterial-Fungal Interactions. Microorganisms 7, 459 (2019).

O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J.M., and Vilgalys, R.: Fungal community analysis by large-scale sequencing of environmental samples. Appl. Environ. Microbiol. 71, 5544-5550 (2005).

Ornelas, A., Dowdell, A.S., Lee, J.S. and Colgan, S.P.: Microbial Metabolite Regulation of Epithelial Cell-Cell Interactions and Barrier Function. Cells 11, 944 (2022).

Panpetch, W., Somboonna, N., Palasuk, M., Hiengrach, P., Finkelman, M., Tumwasorn, S., and Leelahavanichkul, A.: Oral Candida administration in a Clostridium difficile mouse model worsens disease severity but is attenuated by Bifidobacterium. PLoS One 14, e0210798 (2019).

Peleg, A.Y., Tampakakis, E., Fuchs, B.B., Eliopoulos, G.M., Moellering Jr., R.C., and Mylonakis, E.: Prokaryote-eukaryote interactions identified by using Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 105, 14585-14590 (2008).

Pierce, E.C., Morin, M., Little, J.C., Liu, R.B., Tannous, J., Keller, N.P., Pogliano, K., Wolfe, B.E., Sanchez, L.M., and Dutton, R.J.: Bacterial-fungal interactions revealed by genome-wide analysis of bacterial mutant fitness. Nat. Microbiol. 6, 87-102 (2021).

Puccetti, M., Pariano, M., Wojtylo, P., Schoubben, A., Giovagnoli, S., and Ricci, M.: Turning Microbial AhR Agonists into Therapeutic Agents via Drug Delivery Systems. Pharmaceutics 15, 506 (2023).

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D.R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H.B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H, Wang, J., Brunak, S., Dore, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., Meta, H.I.T.C., Bork, P., Ehrlich, S.D., and Wang, J.: A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59-65 (2010).

Roager, H.M. and Licht, T.R.: Microbial tryptophan catabolites in health and disease. Nat. Commun. 9, 3294 (2018).

Romani, L.: Immunity to fungal infections. Nat. Rev. Immunol. 4, 1-23 (2004).

Romani, L.: Immunity to fungal infections. Nat. Rev. Immunol. 11, 275-288 (2011).

Romani, L., Bistoni, F., and Puccetti, P.: Fungi, dendritic cells and receptors: a host perspective of fungal virulence. Trends Microbiol. 10, 508-514 (2002).

Romani, L., Zelante, T., Palmieri, M., Napolioni, V., Picciolini, M., Velardi, A., Aversa, F., and Puccetti, P.: The cross-talk between opportunistic fungi and the mammalian host via microbiota's metabolism. Semin. Immunopathol. 37, 163-171 (2015).

Santus, W., Devlin, J.R., and Behnsen, J.: Crossing Kingdoms: How the Mycobiota and Fungal-Bacterial Interactions Impact Host Health and Disease. Infect Immun 89, e00648-20 (2021).

Seo, S.K. and Kwon, B.: Immune regulation through tryptophan metabolism. Exp. Mol. Med. 55, 1371-1379 (2023).

Smith, M.G., Des Etages, S.G., and Snyder, M.: Microbial synergy via an ethanol-triggered pathway. Mol. Cell. Biol. 24, 3874-3884 (2004).

Sovran, B., Planchais, J., Jegou, S., Straube, M., Lamas, B., Natividad, J.M., Agus, A., Dupraz, L., Glodt, J., Da Costa, G., Michel, M.L., Langella, P., Richard, M.L., and Sokol, H.: Enterobacteriaceae are essential for the modulation of colitis severity by fungi. Microbiome 6, 152 (2018).

Stasi, C., Sadalla, S., and Milani, S.: The Relationship Between the Serotonin Metabolism, Gut-Microbiota and the Gut-Brain Axis. Curr. Drug Metab. 20, 646-655 (2019).

Steffan, B.N., Venkatesh, N., and Keller, N.P.: Let's Get Physical: Bacterial-Fungal Interactions and Their Consequences in Agriculture and Health. J. Fungi (Basel) 6, 243 (2020).

Stockinger, B., Shah, K., and Wincent, E.: AHR in the intestinal microenvironment: safeguarding barrier function. Nat. Rev. Gastroenterol. Hepatol. 18, 559-570 (2021).

Trunk, K., Peltier, J., Liu, Y.C., Dill, B.D., Walker, L., Gow, N.A.R., Stark, M.J.R., Quinn, J., Strahl, H., Trost, M., and Coulthurst, S.J.: The type VI secretion sys-

tem deploys antifungal effectors against microbial competitors. Nat. Microbiol. 3, 920-931 (2018).

Underhill, D.M. and Iliev, I.D.: The mycobiota: interactions between commensal fungi and the host immune system. Nat. Rev. Immunol. 14, 405-416 (2014).

Valentine, M., Benade, E., Mouton, M., Khan, W., and Botha, A.: Binary interactions between the yeast Candida albicans and two gut-associated Bacteroides species. Microb. Pathog. 135, 103619 (2019).

van der Linden, J.W., Camps, S.M., Kampinga, G.A., Arends, J.P., Debets-Ossenkopp, Y.J., Haas, P.J., Rijnders, B.J., Kuijper, E.J., van Tiel, F.H., Varga, J., Karawajczyk, A., Zoll, J., Melchers, W.J., and Verweij, P.E.: Aspergillosis due to voriconazole highly resistant Aspergillus fumigatus and recovery of genetically related resistant isolates from domiciles. Clin. Infect. Dis. 57, 513-520 (2013).

van Leeuwen, P.T., van der Peet, J.M., Bikker, F.J., Hoogenkamp, M.A., Oliveira Paiva, A.M., Kostidis, S., Mayboroda, O.A., Smits, W.K., and Krom, B.P.: Interspecies Interactions between Clostridium difficile and Candida albicans. mSphere 1, e00187-16 (2016).

Venkatesh, M., Mukherjee, S., Wang, H., Li, H., Sun, K., Benechet, A.P., Qiu, Z., Maher, L., Redinbo, M.R., Phillips, R.S., Fleet, J.C., Kortagere, S., Mukherjee, P., Fasano, A., Le Ven, J., Nicholson, J.K., Dumas, M.E., Khanna, K.M., and Mani, S.: Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity 41, 296-310 (2014).

Wagner, R.D., Pierson, C., Warner, T., Dohnalek, M., Farmer, J., Roberts, L., Hilty, M., and Balish, E.: Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. Infect. Immun. 65, 4165-4172 (1997).

Wang, L., Zhang, K., Zeng, Y., Luo, Y., Peng, J., Zhang, J., Kuang, T., and Fan, G.: Gut mycobiome and metabolic diseases: The known, the unknown, and the future. Pharmacol. Res. 193, 106807 (2023).

Wheeler, M.L., Limon, J.J., Bar, A.S., Leal, C.A., Gargus, M., Tang, J., Brown, J., Funari, V.A., Wang, H.L., Crother, T.R., Arditi, M., Underhill, D.M., and Iliev, I.D.: Immunological Consequences of Intestinal Fungal Dysbiosis. Cell Host Microbe 19, 865-873 (2016).

Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C., and Siuzdak, G.: Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc. Natl. Acad. Sci. USA 106, 3698-3703 (2009).

Yang, W., Zhou, Y., Wu, C., and Tang, J.: Enterohemorrhagic Escherichia coli promotes the invasion and tissue damage of enterocytes infected with Candida albicans in vitro. Sci Rep 6, 37485 (2016).

Yano, J.M., Yu, K., Donaldson, G.P., Shastri, G.G., Ann, P., Ma, L., Nagler, C.R., Ismagilov, R.F., Mazmanian, S.K., and Hsiao, E.Y.: Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161, 264-276 (2015).

Zeier, J.: New insights into the regulation of plant immunity by amino acid metabolic pathways. Plant Cell Environ. 36, 2085-2103 (2013).

Zeise, K.D., Woods, R.J., and Huffnagle, G.B.: Interplay between Candida albicans and Lactic Acid Bacteria in the Gastrointestinal Tract: Impact on Colonization Resistance, Microbial Carriage, Opportunistic Infection, and Host Immunity. Clin. Microbiol. Rev. 34, e0032320 (2021).

Zelante, T., Iannitti, R.G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., Zecchi, R., D'Angelo, C., Massi-Benedetti, C., Fallarino, F., Carvalho, A., Puccetti, P., and Romani, L.: Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 39, 372-385 (2013).

Zhang, F., Aschenbrenner, D., Yoo, J.Y., and Zuo, T.: The gut mycobiome in health, disease, and clinical applications in association with the gut bacterial microbiome assembly. Lancet Microbe 3, e969-e983 (2022).