**Microreview**

**Fungal–bacterial interactions and their relevance in health**

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**Summary**

Cross-kingdom interactions between bacteria and fungi are a common occurrence in the environment. Recent studies have identified various types of interactions that either can take the form of a synergistic relationship or can result in an antagonistic interplay with the subsequent destruction or inhibition of growth of bacteria, fungi or both. This cross-kingdom communication is of particular significance in human health and disease, as bacteria and fungi commonly colonize various human surfaces and their interactions can at times alter the outcome of invasive infections. Moreover, mixed infections from both bacteria and fungi are relatively common among critically ill patients and individuals with weak immune responses. The purpose of this review is to summarize our knowledge on the type of interactions between bacteria and fungi and their relevance in human infections.

**Introduction**

Bacteria and fungi are abundant in nature and frequently co-inhabit many natural environments. As a result, fungal–bacterial interactions are ubiquitous in any environment, and their outcome is essential to determine which species will grow and survive. These interactions can take various forms and can be either synergistic or antagonistic. While the variety of different microbial species in the environment far exceed the ones that are relevant to medicine, fungi and bacteria are common colonizers of the respiratory and gastrointestinal tracts of animals, and their interactions are of particular significance in human health and disease (Morales and Hogan, 2010, Peleg et al., 2010). Using various combinations of the terms “fung”, “bacteria” and “interaction”, we performed a review of the literature to identify studies that describe the forms of interaction between bacteria and fungi and their relevance to human infections (Fig. 1).

**Physical interactions**

One of the simplest forms of interactions between prokaryotes and eukaryotes is physical interaction. Indeed, in several cases, bacteria and fungi compete for the same food source or for the same space in a contained and limited environment. A great example of this form of interplay among different species is found in the plant root environment, where the consumption of nutrients from bacterial species prevents the growth of pathogenic fungi (Whipps, 2001). Similarly, in the case of wine fermentation, utilization of trophic sources by Saccharomyces cerevisiae regulates the growth of the lactic acid bacterium Oenococcus oeni and thus controls the occurrence of malolactic fermentation (Alexandre et al., 2004).

An example of more complex physical interactions was recently identified in in vitro experiments where scientists studied mixed biofilms formed by the fungus Candida albicans and bacterial species commonly found in the human gastrointestinal tract (Fox et al., 2014). The researchers found that formation of biofilm by C. albicans encases anaerobic bacteria and promotes their growth by protecting them from oxic environmental conditions. A more recent article showed a similar relationship between the bacteria Burkholderia terrae and several soil fungi. Burkholderia spp. attached to the fungal surfaces and formed biofilm layers and agglomerates, which protected the fungi in the soil from subsequent introduction of antifungal substances (Nazir et al., 2014). On the other hand, different experiments have shown that Pseudomonas aeruginosa bacteria are able to bind to C. albicans hyphae, forming dense biofilms that ultimately lead to the...
destruction of the fungus (Hogan and Kolter, 2002). Moreover, recent studies have evaluated the mechanism of adherence of the bacteria *Staphylococcus aureus* (Peters et al., 2012) and *Streptococcus gordonii* (Silverman et al., 2010) to *C. albicans* hyphae. The investigators have shown that interestingly, the *C. albicans* adhesin agglutinin-like sequence 3 (Als3p) is playing a major role in the adherence process for both bacterial pathogens.

Interactions via secreted molecules

Both bacteria and fungi produce a variety of secreted molecules that influence their environment. Some of these molecules directly influence other microbial species by either promoting or inhibiting their growth, while others indirectly affect them by changing the environmental properties. Secreted fungal molecules that can adversely affect the growth of bacterial species have been the backbone of penicillin-based antimicrobial agents (Houbraken et al., 2011), and the search for novel antibacterial compounds continues to be driven by agents produced by fungal species. For example, researchers recently identified several endophytic fungi whose mycelial extracts have the ability to decrease the growth of *Staphylococcus* spp. (Liang et al., 2012).

Similarly, several bacteria produce secreted molecules that can kill fungal species. Indeed, a recent study showed that phenazines produced by *P. aeruginosa* have the potential to act synergistically with the azole class of antifungals against *Candida* spp. (Nishanth Kumar et al., 2014). Further, Gram-negative bacteria like *Pseudomonas* spp. and *Burkholderia tropica* produce certain volatile compounds that inhibit the growth of *Fusarium* spp. (Cordero et al., 2014). Specifically, *Pseudomonas* spp. have been shown to secrete a quorum-sensing molecule, known as homoserine lactone, which is able to inhibit *C. albicans* hyphal formation (Hogan et al., 2004). Finally, this kind of antagonistic interaction between bacteria and fungi has been proven to be relevant in vivo. In two pivotal studies, researchers using the invertebrate model host *Caenorhabditis elegans* showed that co-infection of the nematode with *Salmonella enterica* and *C. albicans* results in decreased virulence of the fungus (Tampakakis et al., 2009) and this is mediated by the secreted molecule SopB produced by *Salmonella* spp. (Kim and Mylonakis, 2011). In a similar study, Peleg et al. used a *C. albicans–Acinetobacter baumanii* co-infection model in *C. elegans* and proved that when the yeast cells develop a quorum, they can secrete the quorum-sensing molecule farnesol, which is able to inhibit *A. baumanii* growth (Peleg et al., 2008). The importance of the discovery of such compounds is underscored by the constantly rising rates of antimicrobial drug resistance as at least some of these agents may lead to the discovery of new antimicrobial agents, which could be used against human infections.

Conversely, there are cases where certain microbial compounds promote the cross-kingdom growth of other species. A recent example of such an interaction is the production of ethanol by *C. albicans*. Specifically, Chen
et al. discovered that in cases of co-existence of *C. albicans* with *P. aeruginosa*, ethanol production by the yeast up-regulates the transcription of diguanylate cyclase WspR of bacteria, thus leading to bacterial biofilm formation (Chen et al., 2014). A similar interaction where ethanol production by a fungus results in increased bacterial growth, and virulence was observed in an *in vivo* model of *Saccharomyces* spp. and *Acinetobacter* spp. co-infection of the nematode *C. elegans*, where the investigators found that combined infection leads to increased virulence compared with infection with *Acinetobacter* spp. alone, which was at least partially mediated by ethanol production by the fungus (Smith et al., 2004).

**Alteration of host immune response**

A type of polymicrobial interaction with particular relevance in human infections is related to the effect of different pathogens on the host immune response. It has been recently appreciated via studies in invertebrate model hosts that fungi and bacteria induce different innate immune defences (Pukkila-Worley et al., 2011). Therefore, by altering the type of host defence, co-infection with bacterial and fungal pathogens might induce quite different responses than infection with either pathogen alone and therefore alter the outcome of the disease. Indeed, investigators have shown that this is true, at least in mammalian models. For example, in a recent research article, Roux et al. showed that colonization of rat respiratory tract with *C. albicans* increases the risk of development of subsequent bacterial pneumonia by inducing a Th1–Th17 response and increasing the circulating levels of interferon gamma (Roux et al., 2013). In parallel, murine peritonitis caused by both *C. albicans* and *S. aureus* resulted in increased end-organ damage and higher mortality compared with single-pathogen infection, which was mediated by higher levels of circulating inflammatory cytokines (Peters and Noverr, 2013). However, not all polymicrobial interactions result in increased microbial virulence. For example, in a pneumonia model in mice, investigators showed that *Candida* spp. colonization led to the recruitment of natural killer cells and increased levels of cytokine IL-22 and protected the mice from *Pseudomonas* spp. pneumonia (Ader et al., 2011).

**Relevance of fungal–bacterial interactions in human infections**

Cross-kingdom communications between bacteria and fungi play a significant role in human infections. This is an increasingly common conclusion among scientists studying polymicrobial infections or infections of human surfaces that are commonly colonized by other microbial species, like the respiratory and gastrointestinal tracts. It is now evident that in most cases of polymicrobial infections, the outcome and manifestations of the disease are far more complex than what would be expected by the simple additive effect of both pathogens. Indeed, as one can derive from the limited knowledge of the variety of possible inter-kingdom interactions *in vitro* as well as in natural habitats or in model hosts, bacteria and fungi can, depending on the nature of their interplay, enhance or hamper their virulence and growth, provide protection to other species against lethal compounds and change the host response to allow for a completely altered pattern of infection by the other species. It is of no surprise therefore that the pathogenesis and outcomes of mixed infections have become the centre of interest of many researchers in the field of infectious disease and immunology.

The human gastrointestinal tract is an important source of study of inter-kingdom interactions as it is commonly colonized by multiple bacterial and fungal species. For example, research has shown that the presence of *C. albicans* in the oral cavity results in increased growth and biofilm formation by oral streptococci and thus can increase the risk for the development of dental carries (Sztajer et al., 2014). Similarly, a recent study in mice showed that oral candidiasis affects the integrity of the oral mucosa and provides a point of entry for *S. aureus* bacteria to invade the bloodstream and cause a generalized infection with high mortality (Kong et al., 2015). Further, treatment of candidiasis with antifungal agents reversed the phenomenon and protected the mice from bacteremia. A different relationship between bacteria and fungi in human disease is highlighted by the study of lactic acid bacteria. These bacteria commonly colonize the human gastrointestinal tract along with the female reproductive tract. The presence of the bacteria prevents adherence of *C. albicans* in human epithelial cells and thus is able to prevent localized and systemic *Candida* spp. infections (Boris and Barbes, 2000). Therefore, it is not surprising that broad spectrum antibacterial agents that are able to kill these protective bacterial species can promote *Candida* spp. infections (Hogenauer et al., 1998).

Specifically, in the case of the female genitourinary tract, there is now literature suggesting that *Lactobacilli* present in female genital tract has the ability to prime the immune response against fungal pathogens such as *C. albicans* (Rizzo et al., 2013, Zelante et al., 2013) and can also prevent fungal adhesion and growth (Parolin et al., 2015).

More complex and less well-understood interactions between bacteria and fungi take place in the case of respiratory tract infection in individuals colonized with fungal species. This situation is particularly common in the case of ventilator-associated pneumonia (VAP), as the presence of the endotracheal tube creates a favourable...
environment for many microbial species to grow. As discussed earlier, colonization of the respiratory tract by C. albicans has shown conflicting results in the outcomes of subsequent bacterial species in animal models of infection (Ader et al., 2011, Roux et al., 2013). Similarly, observational studies in humans have been inconclusive about whether Candida spp. colonization influences mortality in patients with bacterial VAP, but most studies are in agreement that colonization with Candida spp. is associated with increased morbidity and prolonged hospital stay (Azoulay et al., 2006, Arvanitis et al., 2014). Of note is that a relatively small recent randomized trial showed no benefit in using antifungal agents to treat colonization with Candida spp. in patients with suspicion of VAP (Albert et al., 2014), but given the limited number of participants, further studies are needed before definitive conclusions can be reached.

Finally, interactions between bacteria and fungi can in some cases be of relevance in bloodstream infections. Indeed, an older observational study investigated the outcomes in mixed bloodstream infections with Candida spp. and other bacterial species compared with single bacteremia and found that the presence of Candida spp. in the blood portends poor outcomes (Dyess et al., 1985). Similarly, in a double-blind randomized trial, investigators assessed whether administration of fluconazole empirically in patients with septic shock would alter outcomes by eliminating the sources of concurrent yeast colonization, and found that patients who received the medication had improved 30-day survival compared with the placebo group (Jacobs et al., 2003). Nevertheless, a more recent randomized trial failed to show a survival benefit with preemptive administration of caspofungin in critically ill ventilated patients (Ostrosky-Zeichner et al., 2014).

Conclusions

Our growing awareness of the complexity and multitude of interactions between bacteria and fungi, along with their importance in human health and disease, highlights the need to study these interactions and has brought them into the spotlight of scientific interest. Interactions can take several forms including physical interactions, secreted molecules and alterations of the host immune response and can result in a synergistic or antagonistic relationship between the fungi and the bacteria involved. Understanding the physiology of these cross-kingdom interactions is of profound importance in order to study the pathogenesis of mixed infections as well as mono-infections, which develop in the presence of polymicrobial colonization. Continued research on the field of the pathogenesis of these complex infections is paramount to determine appropriate treatment strategies.

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