Medically important bacterial–fungal interactions

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Abstract | Whether it is in the setting of disease or in a healthy state, the human body contains a diverse range of microorganisms, including bacteria and fungi. The interactions between these taxonomically diverse microorganisms are highly dynamic and dependent on a multitude of microorganism and host factors. Human disease can develop from an imbalance between commensal bacteria and fungi or from invasion of particular host niches by opportunistic bacterial and fungal pathogens. This Review describes the clinical and molecular characteristics of bacterial–fungal interactions that are relevant to human disease.

Gastrointestinal mucositis
Inflammation of the mucosal lining of the gastrointestinal tract (which extends from the oral cavity through to the rectum), often leading to increased microbial transit through the gastrointestinal wall.

Commensal organism
A microorganism that resides on or in the host without causing disease.

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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. Microorganisms have evolved complex mechanisms to promote their survival, defending themselves not only against adverse environmental or nutritional conditions but also against competing organisms. 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 interaction on a contaminated agar plate[1].

This Review will describe the mechanisms and medical importance of bacterial–fungal interactions that occur in or on the human body. Owing to the extensive distribution of the fungal species *Candida albicans* on human skin and mucosal surfaces, this is the fungus that is most frequently implicated in mixed bacterial–fungal infections and will therefore be the main focus of this Review. In addition, the interactions of other fungi such as *Cryptococcus neoformans* with bacteria will be explored.

Sites of bacterial–fungal interactions

In the absence of disease, bacteria and fungi are most commonly found on cutaneous and mucosal surfaces such as the skin, the oral cavity, the gastrointestinal tract and the lower female reproductive tract. Localized insults, such as burn injury to the skin, poor dental hygiene, a surgical procedure, or oral or gastrointestinal mucositis as a consequence of chemotherapy, can lead to disease at these sites, and such diseases are often polymicrobial in nature[2–4] (FIG. 1). Breaches in tissue barriers can also lead to the expansion of these organisms into normally sterile sites such as the bloodstream[5–7]. Similarly, systemic insults to human microbial ecology such as antimicrobial therapy or deficiencies in host immunity can lead to an imbalance in the normal microbial flora and allow a normally benign, commensal organism to become pathogenic, as is the case in vaginal candidiasis, which can occur after the use of systemic antibacterials. Furthermore, colonization of the respiratory tract by bacteria and fungi is especially frequent in patients with chronic lung diseases[8,9] and in mechanically ventilated patients in intensive care units[10,11] (FIG. 1), where mixed bacterial–fungal biofilms are commonly found[12,13]. In-dwelling medical devices that breach the skin can be similarly affected by polymicrobial biofilms[7,14].

Mechanisms of interaction

Bacteria and fungi directly and indirectly influence each other in several ways (FIG. 2). Bacterial factors can influence fungal growth or physiology, and, conversely, fungal factors have been shown to control bacterial behaviour and survival. The virulence of bacteria[15] or fungi[16] can also be influenced by a polymicrobial encounter. Strong evidence indicates that secreted molecules mediate many types of interactions between bacteria and fungi. Interestingly, such extracellular signalling molecules often mediate quorum sensing in single-species communities,

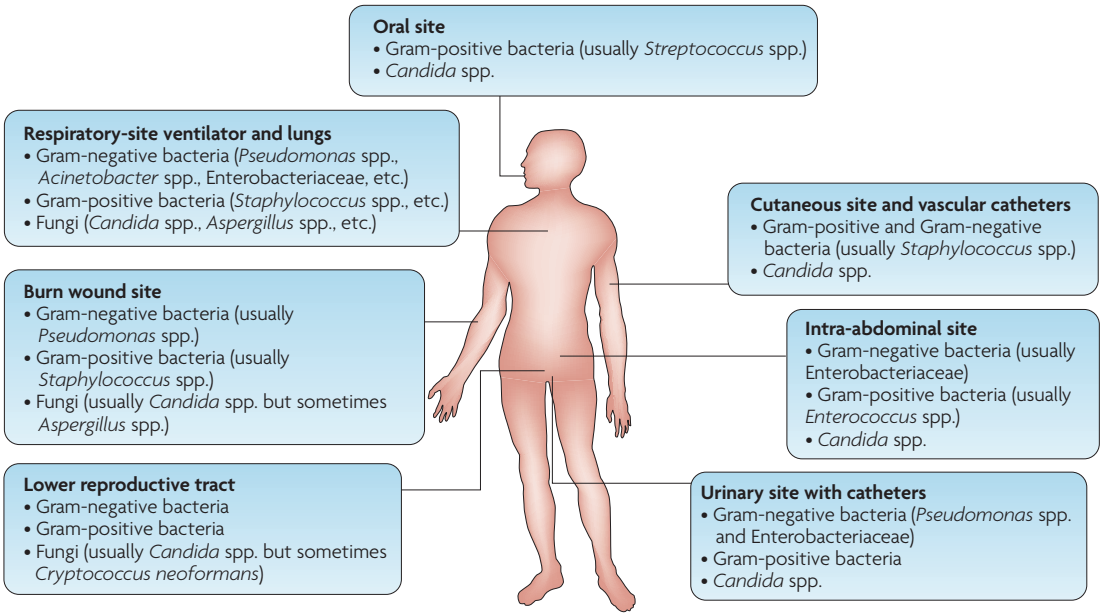


Figure 1 | **Clinically important sites for bacterial–fungal interactions.** Critically ill patients in the intensive-care unit are good examples of the diversity of sites at which bacteria and fungi can interact and cause disease. The boxes describe the organisms that are most commonly found at each site.

suggesting that the effects of one organism on another may be dependent on the population density[15,17–22]. In addition, bacterial toxins such as pseudomonal phenazines have been shown to have antifungal properties[23–26].

Diverse physical interactions between bacteria and fungi have also been described, ranging from bacterial cell contact and aggregation with fungal hyphae or yeast cells[16,27] to organized bacterial biofilms on the surface of fungal hyphae[24] (FIG. 2a). Such cellular interactions have been associated with reduced fungal viability, which may be mediated by bacterial secretion of antifungal molecules into the local environment, by the transfer of toxins directly into the fungal cell through secretion systems or by nutrient depletion. Another mechanism of bacterial–fungal interaction is environmental modification, such as a change in pH[28], which can influence the formation of hyphae in *C. albicans*[29]. In addition to antagonistic interactions, mutually beneficial interactions in mixed-biofilm environments are also possible, whereby the different species may provide protection for each other against an attacking immune response or antimicrobial agent.

Clinical importance

Although data on the clinical relevance of bacterial–fungal interactions are limited, several studies have described the association of bacteria and *Candida* spp. in a range of clinical specimens[4,12,30]. It is not yet clear whether factors such as systemic antibacterial therapy, host immune status or exposure to hospital-acquired pathogens simply predispose a patient to colonization by both bacteria and fungi. However, mixed-species infections can have consequences that differ from those associated with single-species infections. A study of ventilator-associated pneumonia (VAP) caused by *Pseudomonas aeruginosa* suggested that colonization of the respiratory tract with *Candida* spp. may increase the risk of pseudomonal VAP[11]. In support of these observations, other studies have shown that, for individuals with tracheobronchial colonization by *Candida* spp., those who were treated with antifungal drugs had a lower risk of pseudomonal VAP than those who were not[31]. Data from animal models provide additional support for these findings, as described below.

Retrospective human studies suggest that mortality from bloodstream infections that are caused by bacteria or *Candida* spp. ranges from 10–40%[32]. However, very few studies have performed a comparison of single-species and mixed-species infections. One such study identified a poorer survival rate for a mixed bacteria–*Candida* spp. bloodstream infection than for an infection with *Candida* spp. alone[5]. Analyses of the implications of mixed-species infections are limited, as prospective, randomized human trials are rarely possible and observational studies are confounded by the fact that patients with polymicrobial infections may have other risk factors that correlate with a poor clinical outcome, such as greater severity of illness or inadequate therapy against either or both infecting organisms. Furthermore, describing the molecular mechanisms by which any changes in virulence occur in a polymicrobial infection is difficult when studying human disease.

Virulence in animal model systems

An understanding of the pathogenic consequences of bacterial–fungal interactions has come predominately from research using mammalian models of infection (BOX 1). Assessing the changes in virulence in a bacterial–fungal infection is complex and depends on several factors: the temporal association of the microbial

Candidiasis
Infection with a *Candida* species.

Biofilm
A complex community of microorganisms that are often attached to a surface and are surrounded by extracellular matrix.

Quorum sensing
Communication between neighbouring organisms through secreted signalling molecules that allows populations to sense organism density and alter gene expression.

Phenazine
A secreted secondary metabolite and virulence factor of *P. aeruginosa*.

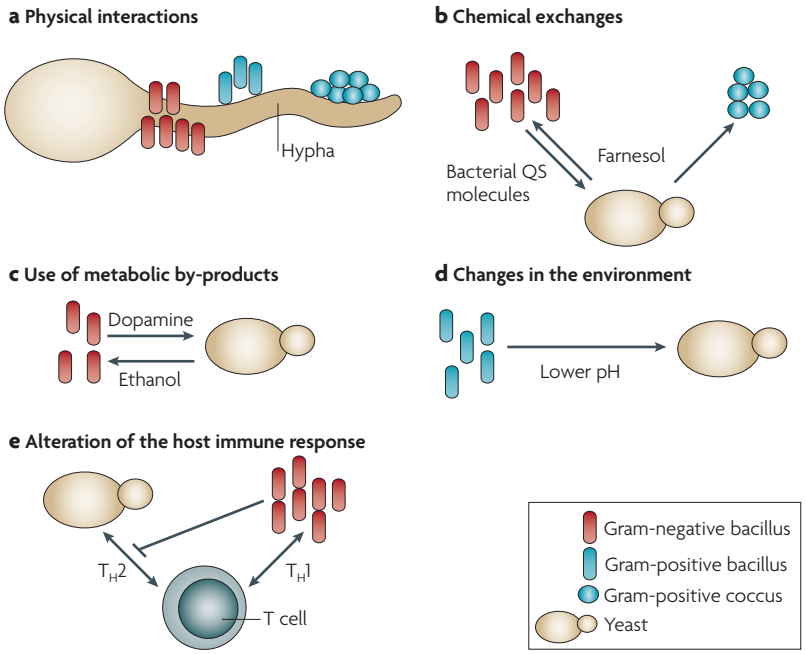


Figure 2 | **Types of bacterial–fungal interactions.** Bacteria and fungi influence each other in many ways and can impact each other's survival or virulence[15,16]. The consequences of these interactions are similarly varied. Such associations can be either beneficial, as in the case of probiotic bacteria that decrease fungal colonization, or detrimental, as can be the case with mixed-species biofilms[16]. **a** | Physical interactions include bacterial attachment to the fungal surface or co-aggregation with fungal cells[16,74,93], the formation of bacterial biofilms on the surface of fungal hyphae[24] and the formation of mixed-species biofilms on an abiotic or host surface[77]. **b** | Chemical exchanges. For example, diverse bacteria produce small molecules that affect the morphology of *Candida albicans*, thereby altering the ability of the fungus to form biofilms or to invade tissues[17,19,22]. **c** | Use of metabolic by-products. **d** | Changes in the environment. **e** | Alteration of the host immune response. QS, quoroum-sensing; T_H, T helper cell.

encounter (whether the bacterial and fungal infections develop simultaneously, or the bacteria develop a quorum before the fungi or vice versa); the site of infection; the size of the inoculum; the organism type; the host; and the outcome measurement of interest. Common outcome measurements for virulence assessment in mammals include the change in the microbial burden at the primary site of infection, the rate of dissemination of the infection from the primary site, the microbial burden at secondary sites, tissue histopathology and host survival over time. Except for host survival, the methods used to study single-species microbial infections can be applied for all of these measurements. Survival-curve analysis and interpretation is more challenging when studying a polymicrobial infection with its single-species comparators and is described in detail in BOX 2.

Enhanced fungal virulence. One of the earliest studies assessing the pathogenic consequences of a mixed bacterial–fungal infection assessed the interaction between *C. albicans* and *Escherichia coli* in mice[33]. Interestingly, attenuation in host killing by *C. albicans* was observed if a sublethal dose of *E. coli* was administered intravenously or intraperitoneally before a lethal dose of *C. albicans*. This mirrored *in vitro* data, which showed that *E. coli* was able to reduce the viability of *C. albicans* over time[33]. However, the mechanism behind these observations was not described. Conversely, if the *E. coli* was given after inoculation with *C. albicans*, enhanced killing was observed (for the most part), which was thought to be mediated by *E. coli* endotoxin[33]. Several other investigators have subsequently had similar results, whereby simultaneous infection with *C. albicans* and *E. coli* caused enhanced killing compared with killing by either organism alone[34–37], and endotoxin seemed to be important for this enhanced virulence[34,35]. These findings have clinical importance, as *E. coli* and *C. albicans* are commensals of the human gastrointestinal tract and are often found in intra-abdominal and hospital-acquired bloodstream infections. These data also highlight the potential importance of adequate antibacterial therapy in the setting of invasive candidiasis.

A further example of the increased virulence of *C. albicans* in the presence of bacteria is shown with a pseudomonal burn wound infection model[38]. Life-threatening candidaemia in human burn victims is often preceded by bacterial infection, especially by *P. aeruginosa*. When burn wounds on mice were pre-infected with a sublethal inoculum of *P. aeruginosa* and then exposed to a sublethal inoculum of *C. albicans*, the mice had a mortality rate of 60%[38]. By contrast, burned mice that were infected with the same inocula individually had a mortality rate of < 10%. Interestingly, microbial burden studies of the burn wound and peripheral organs in co-infected mice showed that the deaths seemed to be due to *C. albicans*. Furthermore, the authors found that the pseudomonal proteolytic enzyme, elastase (LasB; also known as pseudolysin), was responsible for the increased *C. albicans* virulence, but the details of this mechanism were unclear. In a similar study using a rat pneumonia model, investigators found that rats given a subclinical dose of *P. aeruginosa* developed pneumonia only in the presence of viable *C. albicans*[39]. These data support the clinical studies of pseudomonal VAP that were described above[11,31].

A more recent study may shed some light on the possible mechanism behind the enhanced virulence of *C. albicans* in the setting of a bacterial infection. It was found that bacterial peptidoglycan-like molecules found in human serum, known as muramyl dipeptides, act as potent inducers for hyphal development in *C. albicans*[40]. The formation of hyphae is a crucial virulence determinant for *C. albicans* in mammalian infection[41]. The muramyl dipeptides are thought to originate from commensal bacteria, and it is therefore plausible that systemic bacterial infection leads to a large quantity of these hyphae-promoting molecules that subsequently enhance the virulence of *C. albicans*.

Enhanced bacterial virulence. There is also evidence for the enhancement of bacterial virulence by *C. albicans*, illustrating the dynamic nature of polymicrobial infections. This is well described in studies assessing the virulence of mixed *C. albicans* and *Staphylococcus aureus* infection in mice[42,43]. When *S. aureus* alone was inoculated intraperitoneally, no organisms could be

Probiotic
A microorganism that confers a health benefit to the host.

Endotoxin
A toxin that is part of the structure of the bacterium rather than being secreted. In Gram-negative bacteria, it is most commonly lipopolysaccharide in the outer cell membrane.

Box 1 | **Models to study interactions between bacteria and fungi**

Despite an increased appreciation of the importance of polymicrobial encounters and their relevance to human disease, few tools or models exist to study these complex interactions. Important aspects of the bacterial–fungal relationships that would ideally be incorporated into these models include the spatial proximity of the species (that is, the formation of mixed-species biofilms), the influence from environmental conditions, changes in the relationship over time and the host immune response. A number of *in vitro* models have been developed that incorporate these factors to varying degrees[15,19,24,74,86–89]. The benefits of using *in vitro* models for studying the interactions between bacteria and fungi include a highly controlled environment and the ability to screen large numbers of engineered mutants to further characterize the molecular mechanisms of the interaction. However, the disadvantage relates to the absence of a host immune response. Despite mammalian models of infection being logistically, ethically and financially more challenging, the presence of host immune factors is appealing. For the most part, these models are logical adaptations of their use for the study of single-species infections. For example, the standard intravenous or intraperitoneal injection of mice has been used to study polymicrobial bacteraemia or peritonitis, respectively, with *Candida albicans* and *Escherichia coli*[34,37]. Furthermore, standard burn wound and subcutaneous-infection models and a pneumonia model have been used to study mixed infections with *Candida* spp. and *Pseudomonas aeruginosa*[38,39,90]. Mammalian models with specificity for polymicrobial interactions have also been developed, including a model of vaginal candidiasis[91]. This model incorporates not only the host immune responses but also the interactions with commensal microorganisms that are found in a non-sterile site. Most recently, a middle road has been developed with the use of non-mammalian or invertebrate model systems, such as *Caenorhabditis elegans*[16] and *Drosophila melanogaster*[92]. These model systems have the benefit of an innate immune response, are genetically tractable and are easy to work with on a larger scale for screening of mutant strains. Not only can each microorganism be genetically manipulated to help understand the molecular mechanisms of the observed interaction, but the host can also be manipulated to assist in the study of host responses to polymicrobial infections, which is an area of particular interest and also one that is greatly lacking in data.

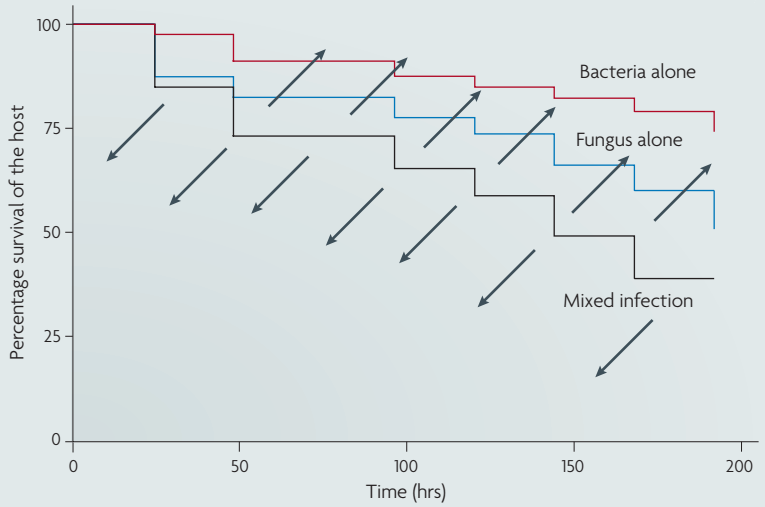
identified in peripheral sites such as the blood, pancreas or spleen and the mice survived. However, when the same dose of *S. aureus* was administered with a sublethal dose of *C. albicans* or with heat-inactivated *C. albicans*, *S. aureus* was recovered 48 hours later in all samples of blood and abdominal organs and most of the mice died[43]. There was no difference observed in the fungal burden of organs for infections with *C. albicans* alone or together with *S. aureus*. Interestingly, histopathology of the peritoneal cavity indicated that *S. aureus* was always found at sites of fungal growth, even when the two pathogens were injected at different sites. Furthermore, in the setting of mixed infection, peripheral organs developed high staphylococcal burdens but negligible amounts of *C. albicans*[43]. These data suggest that *C. albicans* provides protection for *S. aureus* in the peritoneal cavity and enhances its virulence by allowing the bacteria to disseminate to peripheral tissues (a process that would not occur with *S. aureus* infection alone). However, the observed effects may be dependent on the bacterial strain[44]. Similar findings were observed by the same investigators for the Gram-negative bacterium *Serratia marcescens* and for another Gram-positive species, *Enterococcus faecalis*[42], which are both important human pathogens causing hospital-acquired infections that often originate from an intra-abdominal source, as found in the murine model. Given the importance of *S. aureus* as a human pathogen, its ability to cause life-threatening disseminated infections and its common co-habitation with *C. albicans*, these findings are of great importance to our understanding of *S. aureus* pathogenesis in humans. Furthermore, these data also suggest a potential indirect benefit of antifungal treatment in the setting of complex intra-abdominal bacterial–fungal infections: the reduction of bacterial virulence.

Peritoneal cavity
The space in the abdomen that is lined by visceral and parietal peritoneum.

Host responses to mixed infections

Host immune responses to polymicrobial infections have so far been underappreciated. Cross-kingdom microbial interactions such as mixed bacterial–fungal infections challenge the immune system in diverse ways compared with infection with either organism alone. A recent study illustrates the clinical importance of host responses to mixed bacterial–fungal infections[45]. Using a mouse model, the immune responses to direct lung exposure to pseudomonal and/or *C. albicans* cellular lysates were assessed. As had been shown previously, exposure to the fungal antigens led to substantial airway inflammation characterized by eosinophilic infiltration, mucus production and an increase in levels of T helper 2 (T_H2) cell cytokines (namely, interleukin-4 (IL-4), IL-5 and IL-13). Exposure to pseudomonal lysates also led to airway inflammation, but this was characterized by neutrophilic infiltration, no mucus production and an increase in the production of interferon-γ (IFNγ), which is a T_H1 cell $CD4^+$ T cell cytokine[45]. Interestingly, when mice were exposed to both fungal and bacterial lysates, similar degrees of airway inflammation were observed, but the immune response was neutrophilic rather than eosinophilic and T_H1 cell rather than T_H2 cell cytokines were produced[45]. Furthermore, on subsequent exposure to *C. albicans* lysates alone, the immune response had diverted and was characterized by neutrophils and T_H1 cell cytokines. These data demonstrate that, at least in mice, the type of immune response and inflammation that is mounted to a mixed antigen load is dictated by the presence or absence of bacterial antigens. Whether the same results would be obtained using live microorganisms and involving other organ sites is currently unknown. The implications of these findings are broad and lend support to the 'hygiene hypothesis', whereby an increase in

Box 2 | **Survival curves for polymicrobial infections**



Given the complexity of the interactions that can occur in a polymicrobial infection, including those between the pathogens and between the pathogens and the host, it is useful to define the principles of survival-curve interpretation that can be applied to any model of a polymicrobial infection (see the figure). If the host killing by a polymicrobial infection is equivalent to the sum of killing by infection with each pathogen alone (represented by the red and blue lines in the graph), then the killing can be termed 'additive' (represented by the solid black line in the graph). This suggests that the virulence of the two pathogens together is greater than that of either alone, but it may not be due to pathogen–pathogen interactions or changes in host–pathogen interactions; the two pathogens could be killing through independent mechanisms acting over a similar time course. If the host killing by a polymicrobial infection is greater than the sum of killing by infection with each pathogen alone, then the killing can be termed 'synergistic' (represented by the area below the black line in the graph). This implies that the virulence of the two pathogens is not only greater than that of either alone but also greater than that which would be expected if they were killing by independent mechanisms over a similar time course. This pattern suggests a synergistic pathogen–pathogen interaction or a change in host–pathogen interactions that is characterized by increased host susceptibility to one or both of the pathogens. Interpretation becomes challenging when the host killing by a polymicrobial infection is less than additive killing (represented by the area above the black line in the graph). This pattern could have several explanations: that there is an antagonistic interaction between the pathogens, whereby the virulence of one organism is reduced by the other; that the host response to the combined infection is greater or more efficient than the response to infection with either pathogen alone; that the two pathogens mediate killing or virulence through the same pathway, which becomes saturated; or that one pathogen kills more rapidly than the other, preventing the slower pathogen from having any impact on host killing. These simplistic concepts should serve well for the future interpretation of survival curves for polymicrobial infections. Moreover, they highlight the importance of assessing and reporting the virulence end point for the pathogens alone and in combination.

atopic disease in the developed world, mediated by an increase in T_H2 cell responses, is related to a more hygienic environment in which there is less bacterial stimulation[46–48]. Bacterial exposure may reduce such responses through its activation of T_H1 cells (FIG. 2e).

An altered host response to one pathogen may also promote the success of another[39]. In a rat lung model of *C. albicans* colonization and subsequent pseudomonal exposure, an increase in the rate of pseudomonal pneumonia was observed. Interestingly, production of reactive oxygen species by alveolar macrophages was notably inhibited by *C. albicans*[39], suggesting that *C. albicans* may suppress local host immune responses to allow subclinical inocula of *P. aeruginosa* to cause disease.

Atopic disease
A disease associated with an allergy (that is, mediated by immunoglobulin E), such as asthma, eczema and hay fever.

Specific bacterial–fungal interactions

Interactions between* Pseudomonas *spp. and* Candida *spp.

Many studies have described mixed infections with both *P. aeruginosa* and *C. albicans* in cases ranging from contaminated catheters to chronic lung infections[4,8,49,50], and clinical observations suggest that the bacterial and fungal populations influence each other[31,51,52]. *In vitro* studies show the wide spectrum of interactions between *P. aeruginosa* and *C. albicans* (FIG. 3) and emphasize the importance of environmental conditions in determining the outcome of an interaction. For example, in liquid media, *P. aeruginosa* adheres to and forms biofilms on *C. albicans* filaments but not on yeast cells[24] (FIG. 3a). This biofilm formation leads to the death of the fungal cell, brought about by the action of two pseudomonal virulence factors: a secreted haemolytic phospholipase C that degrades phosphatidylcholine (an abundant phospholipid in eukaryotes), and redox-active phenazines, which generate highly toxic reactive oxygen species[23,24]. Although biofilm formation is required for fungal killing in liquid co-cultures[24], the spatial restriction during growth on agar plates demonstrates the toxicity of the phenazines of *P. aeruginosa* towards both yeast and hyphal forms of *C. albicans*[23]. These *in vitro* interactions may reflect the antagonism that is observed between *P. aeruginosa* and *C. albicans* (and other fungi) in chronic infections, as evidenced by the increase in fungal growth on host treatment with antibacterial compounds[52].

C. albicans populations secrete a quorum-sensing molecule called farnesol, which represses hyphal growth despite cues that normally trigger filamentation, such as serum and 37 °C temperatures[53]. Farnesol acts by repressing elements in the Ras1–cyclic AMP–protein kinase A pathway, which positively regulates hyphal growth[18], and effects on other signalling pathways have also been observed. Interestingly, 3-oxo-C_{12}-homoserine lactone, a quorum-sensing molecule produced by *P. aeruginosa*, has a similar effect on *C. albicans*[9,19], and evidence suggests that it acts by a similar mechanism to farnesol[18] (FIG. 3b). Furthermore, other organisms, including *Burkholderia cenocepacia*[17] and *Xanthomonas campestris*[22], produce decenoic acids that similarly repress hyphal growth in *C. albicans*. The finding that *C. albicans* morphology is influenced by secreted factors that are produced by phylogenetically diverse species opens up three important issues. The first issue is whether the production of bacterial molecules like 3-oxo-C_{12}-homoserine lactone influences the behaviour of co-infecting fungal pathogens *in vivo* and whether this effect also reflects how these compounds impact human cells. The second issue is whether these molecules affect other fungi and whether these activities can be exploited to attenuate fungal virulence. The third issue is whether other bacterial pathogens or members of the commensal microflora produce molecules with similar effects on *C. albicans* or other fungi.

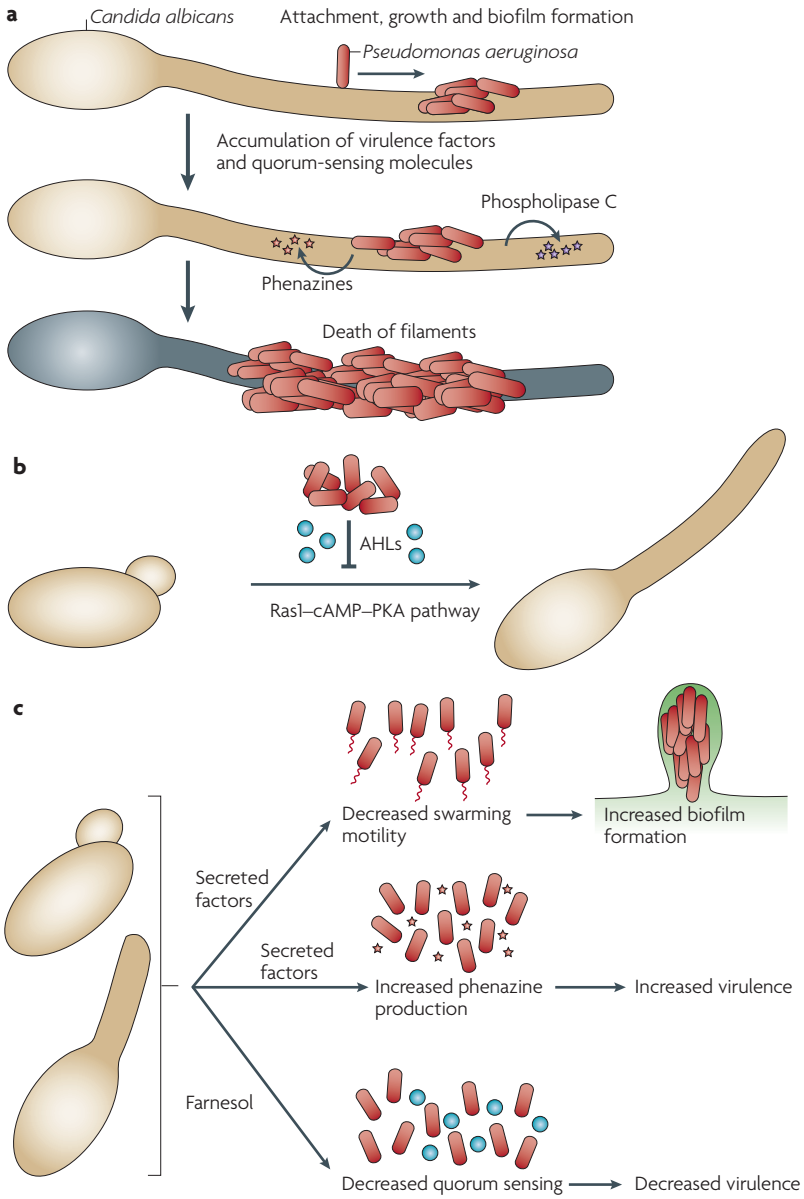


Figure 3 | **Molecular mechanisms of the interactions between *Pseudomonas aeruginosa* and *Candida albicans*. a** | *Pseudomonas aeruginosa* can attach to the surface of *Candida albicans* hyphae (but not yeast cells) and form biofilms[24]. Production of phospholipase C[24] and phenazines[23,94] by *P. aeruginosa* leads to the death of the fungal filament. **b** | In the mixed-species biofilm, quorum-sensing molecules that are produced by both *P. aeruginosa* and *C. albicans* have roles in autoregulation and interspecies communication[9,15,19]. Acyl homoserine lactones (AHLs) such as 3-oxo-C_{12}-homoserine lactone produced by *P. aeruginosa* inhibit the Ras1–cyclic AMP (cAMP)–protein kinase A (PKA) pathway for hyphal growth in *C. albicans*, thereby inhibiting filamentation of the fungus[18]. Because yeast cells have increased survival in the presence of *P. aeruginosa*, the switch to growth as yeast may contribute to the coexistence of both species in mixed infections. **c** | *C. albicans* modulates the behaviour of *P. aeruginosa* through the production of farnesol[15], which alters quorum-sensing regulation. Other uncharacterized *C. albicans* factors increase the production of virulence factors or alter swarming motility and biofilm formation[9,23].

C. albicans also affects *P. aeruginosa* in a range of ways (FIG. 3c). Secreted factors produced by *C. albicans* inhibit swarming motility in *P. aeruginosa*, which may lead to enhanced *P. aeruginosa* biofilm formation on surfaces such as catheters or ventilator apparatus[9,54,55]. Moreover, farnesol alters the regulation of quorum sensing in *P. aeruginosa*[15]. The presence of the fungus may also enhance the production of bacterial virulence factors such as phenazines[23].

Interactions between* Acinetobacter *spp. and fungi. *Acinetobacter* spp. are Gram-negative, non-fermenting, oxidase-negative bacteria that are ubiquitous in the environment[56]. The most clinically important species, *Acinetobacter baumannii*, has evolved to become a highly troublesome pathogen in hospitals worldwide, causing a range of infectious syndromes, including VAP and catheter-related bloodstream infection[56–58]. In health care institutions *A. baumannii* and *C. albicans* commonly co-inhabit ecological niches, including urinary and vascular catheters, ventilator tubing and patient wounds[10,59,60]. In contrast to interactions between fungi and *Pseudomonas* spp., the first interactions between fungi and *Acinetobacter* spp. were only recently described[16,61,62]. Smith *et al.* reported that *Acinetobacter* spp., including *A. baumannii*, exhibited enhanced growth in the presence of *Saccharomyces cerevisiae*, as a result of ethanol secretion by the yeast[62]. Furthermore, in the presence of ethanol, *Acinetobacter* spp. were more resistant to osmotic stress and were also more virulent, as determined by using a *Caenorhabditis elegans* infection model[62]. These data may help to explain why *Acinetobacter* spp. have become so successful in the hospital environment.

In contrast to this synergistic relationship, an antagonistic interaction between the more clinically relevant yeast, *C. albicans*, and *A. baumannii* has been described[16]. It was shown, through the use of a *C. elegans* polymicrobial infection assay, that *A. baumannii* is able to inhibit several important virulence determinants of *C. albicans*, including hyphae and biofilm formation[16] (FIG. 4). Interestingly, the inhibitory effect of *A. baumannii* toward *C. albicans* attenuated the virulence of the fungus, as determined by reduced killing of *C. elegans*[16]. A recent study showed that outer-membrane protein A (OmpA; also known as Omp38) of *A. baumannii* is essential for the attachment of the bacterium to *C. albicans* filaments[63]. Interestingly, this investigation also showed that OmpA is essential for bacterial attachment to mammalian epithelial cells and that apoptotic cell death follows attachment[63].

Highlighting the complexity of polymicrobial infections, *C. albicans* is also capable of mounting a counteroffensive against *A. baumannii*[16]. When *C. albicans* cells are allowed to form a quorum in a biofilm environment, the viability of *A. baumannii* grown in this environment is reduced. As has been shown for other bacteria (FIG. 5), this effect was mediated by farnesol[16].

Other interactions between Gram-negative bacteria and fungi. Numerous microbial interactions occur in the human gastrointestinal tract, and the gut is a common site for *C. albicans* colonization. In addition to the interactions between *C. albicans*, *E. coli* and *S. marcescens*, an interaction between the human

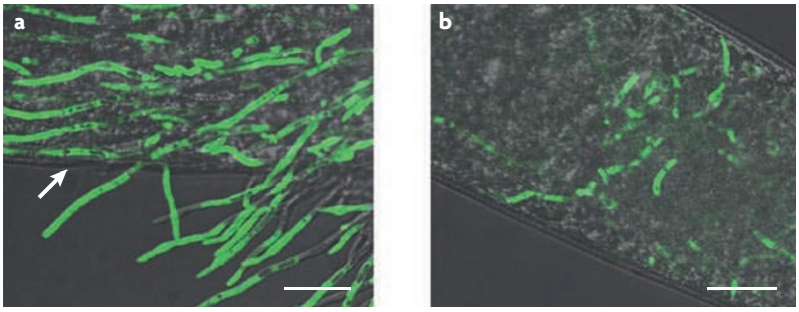


Figure 4 | **Interactions between *Acinetobacter baumannii* and *Candida albicans* in the substitute host, *Caenorhabditis elegans*. a** | When *Caenorhabditis elegans* is infected with the yeast *Candida albicans* and then incubated in liquid medium, the yeast cells build up in the gut of the worm and then undergo a morphogenic transition to filamentous cells (green fluorescent cells)[16]. These filaments penetrate the body of the worm (white arrow), leading to the worm's death. **b** | Interestingly, when the worm is infected with both *C. albicans* and the Gram-negative, pathogenic bacterium *Acinetobacter baumannii*, the number of worms dying with penetrative filamentation is significantly lower. The bacteria inhibit the penetrative *C. albicans* filamentation in the worm. This bacterial antagonism of the fungus leads to reduced virulence of the fungus toward *C. elegans*, as determined by the reduced killing that is seen with a mixed infection. The scale bars represent 20 μm. Images reproduced, with permission, from REF. 16 © (2008) National Academy of Sciences, USA.

gastrointestinal tract pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *C. albicans* has been reported recently[27]. The *C. elegans* polymicrobial infection model was used to study this interaction, as *S.* Typhimurium and *C. albicans* both cause a persistent and lethal gut infection in the nematode[64–66]. As seen with other Gram-negative pathogens, *S.* Typhimurium antagonized *C. albicans* by inhibiting its growth and the formation of filaments and biofilms. The interaction seemed to be mediated by a heat-stable secretory molecule that was produced from late exponential phase onwards. Mutation in an *S.* Typhimurium quorum sensor caused no difference in the observed inhibition of *C. albicans*, and the identity of the active molecule is currently unknown. These data suggest that *S.* Typhimurium uses aggressive techniques to antagonize commensal gastrointestinal organisms for its pathogenesis towards humans.

Another example of a cross-kingdom interaction mediated by a secreted small molecule is that between *Burkholderia cepacia* and *C. albicans*[17]. The bacterium *B. cepacia* is another Gram-negative organism that predominantly infects the lungs of patients who are immunocompromized or who have chronic lung disease, including chronic granulomatous disease and cystic fibrosis. Boon and colleagues identified a novel signalling molecule in *B. cenocepacia* that is a structural homologue of a quorum-sensing molecule and that is known as diffusible signal factor (DSF)[17]. This molecule inhibited germ tube and filament formation in *C. albicans*. Similarly to the signalling molecule 3-oxo-C_{12}-homoserine lactone from *P. aeruginosa*[19], DSF from *B. cepacia* and *B. cenocepacia* was found to be structurally related to farnesol[17]. We are only just beginning to appreciate the degree of conservation between the small signalling molecules that are involved in cross-kingdom interactions and their potential importance to polymicrobial infections and human disease.

Another example of virulence enhancement in the setting of a polymicrobial interaction is the interaction between the bacterium *Klebsiella aerogenes* and the yeast *C. neoformans*[67]. This opportunistic fungus can lead to serious disseminated and central nervous system infections in immunocompromized patients[68]. After *in vitro* co-cultures on agar, it was observed that colonies of *C. neoformans* turned brown in the presence of *K. aerogenes*. This discoloration was due to the presence of melanin, a potent free radical scavenger[69]. Analysis of the *K. aerogenes* culture filtrate identified dopamine as a possible substrate that could be used by *C. neoformans* for melanin production[67]. These studies and the finding that *C. neoformans* can use a bacterial melanin precursor compound[70] suggest that certain bacteria may augment cryptococcal melanization, protecting it from macrophages.

Interactions between oral streptococci and* Candida *spp. Diverse arrays of microorganisms inhabit the oral cavity and are responsible for common oral diseases such as denture stomatitis[2,71,72]. Mixed-species biofilms develop through co-aggregation and are thought to be important for the development of dental plaque and subsequent complications. Oral streptococci, especially *Streptococcus gordonii*, *Streptococcus oralis* and *Streptococcus sanguinis*, adhere well to *C. albicans*[72,73]. This adherence, at least for *S. gordonii*, seems to be mediated through streptococcal cell surface polysaccharide receptors and polypeptide adhesins[74,75]. Interestingly, these streptococcal species are able to adsorb protein components from human saliva (specifically basic, proline-rich proteins) that promote *C. albicans* adhesion[72]. Whether these mechanisms of adhesion have an evolutionary origin and there is some advantage for a mixed bacterial–fungal biofilm is currently unknown. It is plausible that *C. albicans* has adapted receptors to promote its adhesion to surfaces that are coated with saliva, enabling it to colonize and survive in the oral cavity. Recently, *C. albicans* adhesins such as Als1 and Als5 were found to be important for adhesion and aggregation with bacterial cells[76].

Interactions between staphylococci and* Candida *spp. Electron microscopy of a *Staphylococcus epidermidis* – *C. albicans* biofilm on vascular catheter material showed that the bacterium adhered to both morphological forms of the fungus[77]. Interestingly, compared with a single-organism biofilm, the mixed biofilm seemed more resistant to antimicrobials such as fluconazole and vancomycin. It was concluded that the extracellular matrix formed by *S. epidermidis* may protect *C. albicans* from the antifungal agent. Further work is required to understand the relevance of mixed biofilms for the predisposition of fungi to invasive infection and their susceptibility to antimicrobials and host immune defences.

The effects of the *Candida* spp. quorum-sensing molecule farnesol on bacteria are not limited to

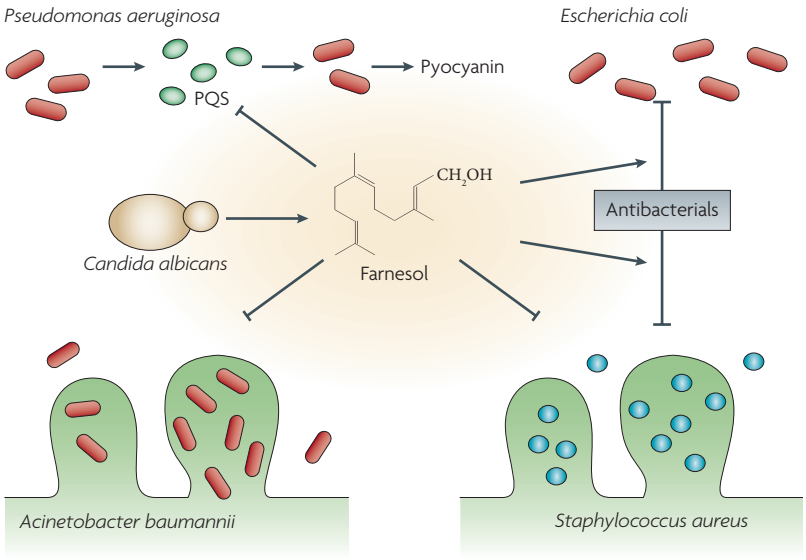


Figure 5 | **Interactions between the *Candida albicans* quorum-sensing molecule farnesol and human pathogenic bacteria.** Farnesol inhibits the *Pseudomonas* spp. quinolone signal (PQS), which is important for quorum sensing in pseudomonal species, and also reduces the production of the pseudomonal virulence factor pyocyanin[15]. Furthermore, farnesol inhibits the viability of *Acinetobacter baumannii* in planktonic and biofilm environments[16] and also increases the susceptibility of *Escherichia coli* to antibacterials[95]. Finally, farnesol is able to interfere with membrane integrity in *Staphylococcus aureus*, reduce the viability of the bacterium, inhibit its ability to form biofilms and increase its susceptibility to antibacterials[20,21,95].

Gram-negative organisms (FIG. 5). Farnesol has also been shown to reduce the viability and biofilm capabilities of *S. aureus*[20,21]. This is thought to be mediated by a disruption in cell membrane integrity, as seen by an increase in ethidium bromide uptake and K^+ loss in the presence of farnesol. Importantly, the susceptibility of *S. aureus* to antibiotics increased in the presence of farnesol[21], presumably owing to cell membrane damage and greater diffusion of antibiotics to target sites.

Interactions between lactobacilli and* Candida *spp. Lactobacilli, which normally inhabit mucosal surfaces associated with the intestinal and female reproductive tracts, have been well studied for their potential to protect against pathogens such as *C. albicans*. The clinical importance of this relationship is highlighted by the complicating effects of systemic antibiotics with activity against lactobacilli, which often lead to vaginitis caused by *Candida* spp. Furthermore, animal model experiments have shown that *in vivo* suppression of *C. albicans* occurs in some cases[78,79]. Many different mechanisms by which lactobacilli could inhibit the growth and virulence of *C. albicans* have been proposed, including the production of hydrogen peroxide or the secretion of organic acids[28,80,81], and there are many reports of *Lactobacillus* spp. with uncharacterized activities against the fungus. Studies using cell culture models have led researchers to propose that some *Lactobacillus* spp. can modulate the virulence of *C. albicans* through bacterial effects on the host immune response[82,83]. Physical interactions between *Lactobacillus* spp. such as *Lactobacillus rhamnosus* and vaginal or cervical epithelial cells or fungi have been observed, and these interactions may contribute to the attenuation of fungal adherence and invasion[78,84,85]. The potential for lactobacilli to play a part in protection against fungal infection is exciting and warrants additional research efforts.

Concluding remarks

Medically important interactions between bacteria and fungi are common. These interactions are highly complex, and the type of interaction that occurs often depends on a range of environmental, pathogen and host factors. Developing appropriate *in vitro* and *in vivo* models to characterize these interactions and their molecular details is imperative for our understanding of their importance to human disease. Furthermore, exploiting the mechanisms that are used by competing microorganisms to antagonize each other could potentially lead to novel treatment options for problematic human pathogens, and this is now, more than ever, a great necessity.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj
Acinetobacter baumannii | *Burkholderia cenocepacia* | *Burkholderia cepacia* | *Caenorhabditis elegans* | *Candida albicans* | *Cryptococcus neoformans* | *Enterococcus faecalis* | *Escherichia coli* | *Lactobacillus rhamnosus* | *Pseudomonas aeruginosa* | *Saccharomyces cerevisiae* | *Salmonella enterica* subsp. *enterica* serovar Typhimurium | *Serratia marcescens* | *Staphylococcus aureus* | *Staphylococcus epidermidis* | *Streptococcus gordonii* | *Streptococcus oralis* | *Streptococcus sanguinis* | *Xanthomonas campestris*
UniProtKB: http://www.uniprot.org
Als1 | Als5 | IFNγ | IL-4 | IL-5 | IL-13 | LasB

FURTHER INFORMATION
Deborah A. Hogan's homepage: http://www.dartmouth.edu/~hoganlab
Eleftherios Mylonakis's homepage 1: http://www2.massgeneral.org/id/labs/mylonakis
Eleftherios Mylonakis's homepage 2: http://chemicalbiology.mgh.harvard.edu/labs-mylonakis.htm

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