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Candida albicans Biofilms and Human Disease

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Abstract

In humans, microbial cells (including bacteria, archaea, and fungi) greatly outnumber host cells. *Candida albicans* is the most prevalent fungal species of the human microbiota; this species asymptomatically colonizes many areas of the body, particularly the gastrointestinal and genitourinary tracts of healthy individuals. Alterations in host immunity, stress, resident microbiota, and other factors can lead to *C. albicans* overgrowth, causing a wide range of infections, from superficial mucosal to hematogenously disseminated candidiasis. To date, most studies of *C. albicans* have been carried out in suspension cultures; however, the medical impact of *C. albicans* (like that of many other microorganisms) depends on its ability to thrive as a biofilm, a closely packed community of cells. Biofilms are notorious for forming on implanted medical devices, including catheters, pacemakers, dentures, and prosthetic joints, which provide a surface and sanctuary for biofilm growth. *C. albicans* biofilms are intrinsically resistant to conventional antifungal therapeutics, the host immune system, and other environmental perturbations, making biofilm-based infections a significant clinical challenge. Here, we review our current knowledge of biofilms formed by *C. albicans* and closely related fungal species.

Keywords

fungi; pathogen; microbiota; microbial community; infection; transcriptional regulation

INTRODUCTION

Biofilms are the predominant growth state of many microorganisms. Over the last 20 years, the definition of biofilm has evolved from a stringent set of specific criteria to a broader description of microbial community structures observed in both natural and laboratory environments. In the most general sense, a biofilm is a community of adherent cells with properties that are distinct from those of free-floating (planktonic) cells (91, 92, 111). Although biofilms are often attached to solid surfaces, they can also form in other

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environments, for example, liquid-air interfaces. A near-universal characteristic of biofilms, compared with their free-floating counterparts, is the greater resistance of their cells to chemical and physical insults (31, 32).

It is now recognized that, for many microorganisms, the biofilm state is probably more relevant to their "natural" settings than to suspension cultures. To name just a few, these settings include aquatic environments (e.g., intertidal sediments that are home to cyanobacteria biofilms), artificial structures (e.g., pipelines where algal biofilms flourish), biomaterials (e.g., implanted heart valves where *Staphylococcus epidermidis* biofilms develop), plant tissues (e.g., root legumes colonized by rhizobia biofilms), and mammalian tissues (where *Candida albicans* biofilms are frequently found). Local effects of biofilms on their environments and/or hosts are complex; they may be favorable, harmful, or benign, and these effects can change over time. The National Institutes of Health estimate that biofilms are responsible, in one way or another, for over 80% of all microbial infections in the United States (61). In this review, we focus on *C. albicans* biofilms, which colonize implanted medical devices and mucosal surfaces, from which they can seed systemic infections in humans (46, 47, 64, 89, 97, 161, 172).

C. albicans is one of the very few fungal species causing disease in humans—millions of others do not. It is a member of the healthy microbiota, asymptomatically colonizing the gastrointestinal (GI) tract, reproductive tract, oral cavity, and skin of most humans (1, 64, 87, 97, 99). In individuals with healthy immune systems, *C. albicans* is often harmless, kept in balance with other members of the local microbiota. However, alterations in the host microbiota (e.g., due to antibiotics), changes in the host immune response (e.g., during stress, infection by another microbe, or immunosuppressant therapy), or variations in the local environment (e.g., shifts in pH or nutritional content) can enable *C. albicans* to overgrow and cause infection. These infections range from superficial mucosal and dermal infections, such as thrush, vaginal yeast infections, and diaper rash, to hematogenously disseminated infection with sizable mortality rates (approaching 40% in some cases) (21, 148, 202). *Candida* infections are especially serious in immunocompromised individuals (such as those with AIDS or those undergoing anticancer or immunosuppression therapies) and healthy people with implanted medical devices (96, 201).

C. albicans produces highly structured biofilms composed of multiple cell types (i.e., round, budding yeast-form cells; oval pseudohyphal cells; and elongated, cylindrical hyphal cells) encased in an extracellular matrix (23, 61, 158, 161). Accounting for 15% of all hospital-acquired sepsis cases, species within the CTG clade (predominantly *C. albicans*, but including several closely related species as well) are the fourth most frequent cause of bloodstream infections in clinical settings and are the predominant fungal species isolated from medical device infections (43, 152, 202, 204). Urinary and central venous catheters, pacemakers, mechanical heart valves, joint prostheses, contact lenses, and dentures are all susceptible to *C. albicans* biofilms (22, 45, 90, 175). Once it forms on an implanted medical device, a *Candida* biofilm has the potential to seed disseminated bloodstream infections and to lead to invasive systemic infections of tissues and organs. Over five million central venous catheters are placed each year in the United States (data obtained from 1992 to 2001) (90, 129). Currently—even with recently improved clinical approaches—biofilm infection occurs

in over 50% of these catheters. Responsible for an estimated 100,000 deaths and $6.5 billion in excess expenditure annually in the United States alone, these infections have serious health and economic consequences. Because fungal biofilms are largely resistant to current antifungal drugs, high antifungal doses together with removal of the colonized medical device are generally required to treat infections (6, 29, 104, 112, 121). Removal of some devices (e.g., artificial heart valves and joints) is costly and, in some cases, dangerous, and administration of high doses of antifungal agents (typically given intravascularly) can cause complications, including kidney and liver damage. Oftentimes, these treatments are not possible, as many critically ill patients are unable to tolerate them.

IN VITRO AND IN VIVO DEVELOPMENT OF *C. ALBICANS* BIOFILMS

C. albicans biofilm formation in the laboratory can be readily observed on a solid surface, for example, on silicone, the common material used for intravascular catheters. Typically, a small silicone square is added to a culture of *C. albicans* (in any of a wide variety of growth media). An initial incubation is performed to allow the *C. albicans* cells to adhere to the silicone square; nonadherent cells are then washed away, and a biofilm is allowed to form. Throughout the process, the cells are constantly shaken to prevent settling; alternatively, a continuous flow of liquid across the biofilm can be imposed, mimicking exposure of an implanted catheter to blood flows. A mature biofilm typically forms within 24 hours and can be visualized by the unaided eye as a cloudy surface structure on top of the silicone square and with a microscope as an organized collection of different cell types. *C. albicans* biofilm formation comprises four temporal stages (10, 23, 47, 75, 142, 194) (Figure 1): (*a*) adherence to a surface (spherical yeast cells), (*b*) proliferation to form a basal layer of anchoring cells, (*c*) growth of pseudohyphae (ellipsoid cells joined end to end) and hyphae (chains of cylindrical cells) concomitant with production of extracellular matrix material, and (*d*) slow dispersal of yeast-form cells from the biofilm to seed new sites. In the laboratory, *C. albicans* biofilms can develop on several substrates and in many types of media, indicating an inherent robustness of biofilm development to changes in external conditions.

In vitro biofilm formation has, for the most part, correlated well with in vivo and ex vivo biofilm models. For example, *Candida* biofilms obtained from patients with denture stomatitis and from patients with infected intravascular catheters confirm the presence of yeast, hyphae and extracellular matrix (114, 162). Biofilm architectures in rat and rabbit central venous and indwelling urinary catheter models and in rat denture stomatitis models also include numerous yeast cells in the basal region, and hyphae and extracellular matrix extending throughout the biofilm (5, 84, 131, 134, 166, 173, 199). Vaginal mucosal mouse models, both in vivo (live mice inoculated with *C. albicans* on the vaginal mucosa) and ex vivo (vaginas excised from euthanized mice and then inoculated with *C. albicans* in tissue culture plates), show similar biofilm architectures, with clear yeast, hyphae, and extracellular matrix evident throughout the biofilms formed on top of the mucosal layers (72). Other animal models for monitoring biofilm formation include rodent oral mucosal, oropharyngeal, subcutaneous, and burn wound models (28, 44, 53, 166). Development of newer systems is under way to visualize the temporal and spatial progression of biofilm infections in live animals using bioluminescence imaging. For example, a vulvovaginal

candidiasis model using a codon-optimized *C. albicans* luciferase bioreporter was used to observe biofilm formation in real time in the vaginal lumen (48). Other *C. albicans* bioluminescent biofilm models include oropharyngeal, cutaneous, subcutaneous, and implanted catheter models (52, 124, 153, 197, 198).

GENETICS OF *C. ALBICANS* BIOFILM FORMATION

Although *C. albicans* is not genetically tractable in the conventional sense (its parasexual cycle is cumbersome to use in the lab), its genome is relatively simple to alter using recombinant DNA technologies. For example, nearly 1,000 gene knockout mutants of this diploid organism have been constructed (out of ~6,000 genes total), and many of these deletion mutants have been screened for biofilm formation. Other approaches include genome-wide transcriptional profiling and proteomics techniques to identify genes and proteins expressed specifically in biofilms (115, 191, 209). These studies have revealed that many hundreds of mRNAs and proteins are differentially expressed between biofilms and planktonic cells.

In this section we review these genetic studies with particular emphasis on the master regulators that orchestrate biofilm formation as well as some of the important nonregulatory genes that have been genetically validated to play important roles in biofilm formation. We exclude genes whose deletions cause broad phenotypes (such as slow growth), as their effects on biofilm formation are likely to be indirect. Because the available deletion libraries are enriched for knockouts of transcriptional regulators, we probably know more about the transcriptional regulation of biofilm production than we do about the process itself. Based on the current literature, we count 50 transcriptional regulators (Table 1) and 101 nonregulatory genes (Table 2) that have functionally validated roles in biofilm formation.

Regulatory Control of *C. albicans* Biofilm Development

In 2012, a large transcriptional network that controls the development of *C. albicans* biofilms was described (140). This network consists of six master transcriptional regulators (Efg1, Tec1, Bcr1, Ndt80, Brg1, and Rob1), each of which is required for biofilm development in both in vitro and in vivo rat catheter and rat denture models (140). Each master regulator also controls the expression of the other master regulators, resulting in a complex, intertwined regulatory network. Together, these six master regulators directly bind to the promoters of and very likely regulate the expression of approximately 1,000 target genes, some of which are additional transcriptional regulators. Work carried out in both planktonic and biofilm conditions has indicated that various target genes play roles in hyphal formation, adhesion, drug resistance, and matrix production (see 61 for a summary), all of which are important characteristics of biofilms. However, the majority of newly identified target genes in the biofilm network have not yet been studied; many have no overt sequence similarity to any previously characterized genes from any organism. Orthology mapping indicates that the entire set of target genes is significantly enriched for “young” genes, suggesting that the ability of *C. albicans* to form biofilms evolved relatively recently with respect to evolutionary timescales. This inference provides an explanation as to why *C. albicans* and closely related species are only a few of the many fungal species able to form

biofilms within a mammalian host. With the outline of the biofilm network (which is undoubtedly incomplete), it is now possible to systematically study the role of the nonregulatory target genes in biofilm development.

In addition to the 6 master transcriptional regulators discussed above, another 44 transcriptional regulators have been identified; if any of these is deleted, some aspect of *C. albicans* biofilm formation is affected under at least one condition (Table 1; revised from 61 and 18). The majority of these regulators are directly bound by at least one of the 6 master biofilm network regulators, indicating that they have direct regulatory connections to the core biofilm circuit (Table 1).

The transcriptional network controlling biofilm development in *C. albicans* may seem overly complex, but it is typical of many other transcriptional networks, particularly those in eukaryotic organisms. For example, mammalian stem cell maintenance, *Drosophila* eye development, and *Arabidopsis* circadian clock rhythms are all controlled by multiple transcriptional regulators that regulate each other and many additional target genes (3, 110, 203, 206). Complex transcriptional circuits also control pseudohyphal growth and the response to osmotic stress in baker's yeast (19, 137) as well as the white-opaque cell-type switch in *C. albicans* (76). Although its significance in any transcriptional network is not fully understood, this high degree of complexity does seem to be a common feature of networks that coordinate morphological changes. In the following sections, we combine genetic results with genomic analyses and break down the biofilm circuit into smaller pieces, each of which contributes to biofilm formation and maintenance.

Adherence

The ability of cells in a biofilm to adhere to each other and to surfaces, which can be hard (a medical device) or soft (a mucosal layer), is important for all stages of *C. albicans* biofilm development. For example, the biofilm master regulator Bcr1 and some of its downstream targets, including the cell wall proteins Als1, Als3, and Hwp1, are all required for adherence during biofilm formation (23, 138, 141, 143, 145, 212). Many additional transcriptional regulators have been implicated in adherence (49, 57, 123, 143, 212; Table 1), the majority having been discovered in a study that screened a library of transcriptional regulator mutants using an in vitro flow cell assay (57). Here, 30 transcriptional regulators were identified as important for adherence to a silicone substrate under these conditions. Of these 30, 4 (Bcr1, Ace2, Snf5, and Arg81) were also required for biofilm formation under common conditions (shaking in microtiter plates) for in vitro biofilm formation. It is clear that biofilm formation by *C. albicans* can occur over a broad range of conditions and that the genetic requirements likely vary from one condition to the next.

Hyphae

C. albicans is distinguished from many other fungal species by its ability to form both yeast cells and hyphae under many different environmental conditions. (This is the basis of the early classification of *C. albicans* as dimorphic.) Although they readily form in planktonic cultures, hyphae are an important structural component of *C. albicans* biofilms; thus, it is not surprising that genes required for hyphal growth in suspension cultures are also necessary

for proper biofilm formation. These include the transcriptional regulators Efg1, Tec1, Ndt80, and Rob1 (140, 163, 174). The hyphae in biofilms contribute to the overall architectural stability of the biofilm, acting as a support scaffold for yeast cells and other hyphae. Thus, the ability to form hyphae and the ability of these hyphae to adhere to one another and to yeast cells are critical for normal biofilm development and maintenance. Indeed, the master regulator Bcr1 is not needed for hyphal formation per se, but it is needed for the hyphae to adhere to one another in biofilms (141).

Extracellular Matrix

Mature *C. albicans* biofilms are encased in a complex material known as the extracellular matrix. Two known transcriptional regulators of biofilm matrix production in *C. albicans* are Rlm1 and Zap1. Deletion of *RLM1* causes a reduction in matrix levels (136), whereas deletion of *ZAP1* leads to an increase in the accumulation of extracellular matrix material, probably by upregulating the glucoamylases Gca1 and Gca2 (144).

Dispersal

Dispersal of cells within a *C. albicans* biofilm into the environment is another important stage in the biofilm life cycle. Cells are dispersed continuously throughout biofilm formation, and they are thought to be primarily (if not exclusively) in the round yeast form (194). Although dispersed cells morphologically resemble yeast cells in the planktonic mode of growth, they have distinct characteristics. These include (relative to planktonic cells) increased adherence properties, the ability to form biofilms more efficiently, and enhanced virulence in mouse models of infection (194). Two transcriptional regulators of *C. albicans* biofilm dispersal have been identified, Nrg1 and Ume6; transcriptional overexpression of either regulator increased the number of dispersed cells actively released from the biofilm (194, 195). Nrg1 probably acts through a Set3 chromatin-modifying complex (77, 139). Indeed, mutant strains of individual Set3 complex members formed extra strong biofilms that were incapable of normal biofilm dispersal and especially recalcitrant to mechanical perturbation (139). Nrg1 and Set3 complex mutants are hyperfilamentous, consistent with their inability to generate yeast-form cells (77, 195).

The molecular chaperone Hsp90 has also been implicated in *C. albicans* biofilm dispersal, as depletion of Hsp90 markedly reduces the number of dispersed cells from a biofilm (167). Depletion of Hsp90 also induces filamentation by relieving Hsp90-mediated repression of the cAMP-PKA signaling pathway (181). The cell wall protein Ywp1 is also important for biofilm dispersal, as deletion of *YWP1* leads to decreased biofilm dispersal and increased biofilm adhesiveness (69, 70). It is possible that any mutation that favors filamentous cells over yeast-form cells reduces biofilm dispersal. Thus, both types of cells are required for fully functioning biofilms.

Drug Resistance and the Extracellular Matrix

The inherent resistance of *C. albicans* biofilms to antimicrobial agents is a key feature of the biofilm mode of growth. This resistance is due to the upregulation of efflux pumps, the presence of the extracellular matrix, and the presence of recalcitrant persister cells. In *C. albicans*, two major classes of efflux pumps modulate drug exportation: the ATP-binding

cassette transporter superfamily (including *CDR1* and *CDR2*) and the major facilitator class (including *MDR1*) (4, 33, 156). In planktonic cells, these efflux pumps are typically upregulated in response to antifungal drugs; in biofilms, however, they become upregulated within the first few hours of surface contact and remain upregulated throughout biofilm development, whether or not an antifungal drug is present (119, 126, 133, 140, 156, 205). This seemingly automatic upregulation of efflux pumps clearly contributes to the recalcitrance of biofilms to treatment with antifungal agents; it may be a manifestation of small-molecule warfare between *C. albicans* and other microbial species that occupy the same environmental niches.

Secreted extracellular matrix also contributes to biofilm drug resistance, both acting as a physical barrier to drug penetration and directly contributing to the overall structural integrity of the biofilm (2, 11, 130, 135). One known constituent of the biofilm matrix that contributes to its drug-resistance properties is the polysaccharide β-1,3-glucan (130). Thus, treatment of biofilms with β-1,3-glucanase increases the susceptibility of biofilms to fluconazole (130), and addition of exogenous β-1,3-glucans increases the tolerance of planktonic cells to fluconazole (122). Genes involved in production and delivery of β-1,3-glucans include the synthase-encoding gene *FKS1* and the genes *BGL2, XOG1*, and *PHR1*, which encode proteins involved in the modification and transport of β-glucans (132, 189). The transcriptional regulator Rlm1 (discussed above) contributes to antifungal drug resistance by regulating the expression of *FKS1* (136). Finally, extracellular DNA is also a component of the matrix in *C. albicans* and contributes, probably indirectly, to drug resistance (117). For example, one study reported that treatment of biofilms with DNAse enhanced the activity of caspofungin and amphotericin B in disrupting mature *C. albicans* biofilms (116).

Understanding how the matrix contributes to drug resistance in *C. albicans* has been challenging, in part because of difficulties in identifying the key matrix constituents. Significant progress has been made in comprehensive analysis of its macromolecular content (209). The *C. albicans* biofilm matrix is predominantly composed of proteins and glycoproteins (55%), carbohydrates (25%), lipids (15%), and nucleic acids (5%). Over 500 proteins were identified in the matrix. Most of these were predicted to be enzymes, including hydrolyzing enzymes, suggesting that the matrix may play an active role in breaking down biopolymers. It is intriguing to consider the biofilm matrix as an extracellular, enzymatically active element of a *C. albicans* biofilm—one that can break down molecules both as a protective response and to access a nutrient source. Polysaccharides make up the second major fraction of the matrix, consisting largely of mannan-glucan complexes made predominantly of α-1,6-linked mannan and α-1,2-linked side chains complexed to β-1,6-glucan (209). Thus, although β-1,3-glucans contribute to drug resistance and are the major cell wall polysaccharide (130), we now know that they constitute only a small fraction of the total polysaccharides present, implying that the matrix is not constructed simply of released cell wall constituents.

Persister Cells

Persister cells are another contributor to the drug-resistance properties of biofilms. A minor subset of metabolically dormant yeast cells that stochastically arise as phenotypical variants within biofilms, persister cells are extremely resistant to antifungal drugs (100). Although we know little about the formation and roles of persister cells in *C. albicans* biofilms, we do know that the drug resistance of persister cells is independent of cell membrane composition and efflux pump expression; rather, it is the result of the metabolically dormant state of the cells (88, 100). We know little about how persister cells are regulated and controlled, despite their importance to the drug resistance of *C. albicans* biofilms.

BIOFILM FORMATION BY OTHER ASCOMYCOTA SPECIES

As mentioned, *C. albicans* is not the only fungal species that can form biofilms in a mammalian host. The closely related species *Candida dubliniensis*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* have all been implicated in biofilm-associated infections (73, 157, 187). Each of these species can form a biofilm in vitro, but the thickness, strength, and robustness in different environmental conditions decrease as species diverge phylogenetically away from *C. albicans*. Thus, among the CTG clade, *C. albicans* is probably the best biofilm former in the sense that it can form relatively thick biofilms under many different environmental conditions. It is also the most extensively studied.

Outside the CTG clade, numerous fungal species have been reported to form biofilms in vitro and in vivo. It should be emphasized that biofilm formation is often loosely defined; in some cases, simply adhering to plastic or glass is considered a sufficient criterion. The type of biofilm formed by *C. albicans* is probably unique to the CTG clade; given that the *C. albicans* biofilm circuit is a recent evolutionary innovation, it is likely that biofilms from more distantly related fungal species were also relatively recent innovations that independently evolved along those clades. Thus, it is unlikely that biofilms from fungal species hundreds of millions of years distant from *C. albicans* are similar in detail. Below, we briefly survey the literature on biofilm formation outside of the CTG clade.

C. albicans is a member of the phylum Ascomycota, which includes the genera *Acremonium, Aspergillus, Blastomyces, Blastoschizomyces, Candida, Cladosporium, Coccidioides, Fusarium, Histoplasma, Paracoccidioides, Pneumocystis, Saccharomyces* and *Scedosporium*. For some of these genera, biofilm formation has been implicated in pathogenesis in humans, but for others this connection is less clear. For example, a number of these biofilm formers (Table 3) have been tested for drug susceptibility in vitro, and cells in biofilms have been found to be more resistant to drugs compared with cells grown in suspension culture. These include (in addition to *Candida*) members from Acremonium, *Aspergillus, Cladosporium, Fusarium*, and *Pneumocystis* (9, 20, 35, 42, 79, 160, 176). Candida glabrata (which, despite the name, lies well outside the CTG clade phylogenetically) forms thin biofilms in vitro (without hyphal cells) on biotic and abiotic surfaces associated with the human host (157).

Saccharomyces cerevisiae, or baker's yeast, rarely causes infections in humans and is generally not considered a pathogen. In a few case studies, however, *S. cerevisiae* has been

implicated in catheter-associated infections with mixed-species biofilms in patients in intensive care units (ICUs), and it is able to form a thin biofilm consisting of round, budding yeast-form cells and pseudohyphal cells in vitro (26, 50, 128, 164). *Blastoschizomyces capitatus* is another rare cause of infection in humans, although more cases have been emerging in highly immunocompromised patients in ICUs. *B. capitatus* can cause catheter-associated bloodstream infections and has the capacity to form a biofilm both in vitro and in vivo (16, 38).

Aspergillus is a fungal pathogen of great medical importance that can form biofilms both in vitro and in vivo (125, 159). *Aspergillus fumigatus* is a common invasive colonizer of the respiratory tract in individuals with cystic fibrosis or other conditions that compromise the immune system (176). During infection, airborne spores enter the host via the airway and undergo a morphological switch to the filamentous growth form upon contact with host tissues. *A. fumigatus* then continues to proliferate along the lining of the respiratory tract, forming a dense network of hyphal cells. Aspergillomas, or dense hyphal balls, may also form as *A. fumigatus* continues to proliferate in the respiratory tract; these structures share many architectural characteristics with biofilms formed by other fungal species (159).

Other Ascomycota members also infect the respiratory tract of mammals. For example, *Pneumocystis jirovecii* causes frequently fatal pneumonia in mammals. Morphological examination of lung tissue infected by *P. jirovecii* reveals a biofilm-like growth structure consisting of round yeast-form clusters that can be recapitulated in vitro under certain laboratory conditions (35, 36). *Scedosporium prolificans*, a recently emerging human fungal pathogen of the respiratory system, may also form biofilms, given that fungus balls, resembling biofilm structures, have been observed in some clinical cases (30). *Histoplasma capsulatum* is another opportunistic pathogen that infects the respiratory system of mammals, causing histoplasmosis (85). *H. capsulatum* typically exists in a filamentous form in the environment outside of the host but is usually found as yeast-form cells within the host. There is at least one report of *H. capsulatum* forming biofilms consisting of dense clusters of yeast-form cells in vitro, suggesting that *H. capsulatum* may exist in biofilms in vivo (154). Along with *H. capsulatum*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, and *Blastomyces dermatitidis* are all sources of mycoses in immunocompromised individuals and have also caused endemic outbreaks in specific geographical regions (62, 113). All infect the respiratory system but are capable of causing invasive infections. Although rare, *C. immitis* has been reported to form biofilms on medical devices (40). Whether *B. dermatitidis* and *P. brasiliensis* form biofilms in vitro or in vivo is unknown.

Finally, the ascomycete species *Fusarium solani*, *Acremonium implicatum*, and *Cladosporium sphaerospermum* can cause keratitis in mammals, a serious infection of the cornea of the eye, as well as other less-common infections. Keratitis is generally thought of as a biofilm infection and has been associated with the use of contact lenses. In fact, a *Fusarium* keratitis outbreak occurred in the United States as a result of a contact lens multipurpose solution lacking sufficient antifungal efficacy against *F. solani* (24, 105). Recent work in vitro has shown that *F. solani*, *A. implicatum*, and *C. sphaerospermum* can all form biofilms under laboratory conditions (211). Though biofilm formation has been most extensively studied for *C. albicans*, it is clear—even from this brief discussion—that

biofilm formation by an evolutionarily broad range of fungal species has enormous impact on human health.

MULTISPECIES BIOFILMS WITH *C. ALBICANS*

Microbial infections are often thought of, and treated, as if a single microbial species were acting alone. Yet they occur in the presence of the human microbiota—the collection of microbes that inhabit the body. This microbiota includes members from three major branches of life: bacteria, archaea, and fungi. Outnumbering human cells by at least a factor of ten, these microbes form a complex ecosystem, one whose balance can be affected by the host's diet, genetic background, and alterations in immunity and by transient environmental perturbations, such as changes in pH, viscosity of mucosal layers, and the use of broad-spectrum antibiotics. Thus, expanding our understanding of biofilm formation in the context of other microbial members is an important goal. Although *C. albicans* is the fungal pathogen most frequently isolated from human infections, other *Candida* species have been found together with *C. albicans* in polymicrobial biofilms from patients (27). These species include *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. guillermondii*, *C. krusei*, and *C. glabrata* (80, 90, 118, 151). We know virtually nothing about the interactions among these *Candida* species in polymicrobial biofilms.

However, limited progress has been made in understanding the dual-species biofilms *C. albicans* forms with a few common bacterial species likely to interact with it in humans. Several studies have investigated such dual-species biofilms comprising *C. albicans* and a bacterium commonly isolated from denture stomatitis, periodontitis, or dental caries, such as *Streptococcus mutans*, *Streptococcus gordonii*, *Actinomyces viscosus*, or *Fusobacterium* species (12, 81, 82). *C. albicans* also interacts with several bacterial species found in the gut, such as *Enterococcus* and *Escherichia* species; those found in the vagina, such as *Lactobacillus* species; and those found in the lungs of patients with cystic fibrosis, such as *Pseudomonas aeruginosa* (13, 34, 54, 78). From these studies, we have learned that *C. albicans* and bacteria can interact with each other by secretion of signaling molecules that influence the behavior of one species toward the other, by direct cell-cell physical contact, and by alterations of the local environment that influence the other species (e.g., alterations in pH and oxygen concentrations). For example, *P. aeruginosa* secretes a 12-carbon acyl homoserine lactone that modulates hyphal growth by *C. albicans* (78). Another study examined dual-species biofilms *C. albicans* forms with one of five prevalent members of the human gut microbiota: *Bacteroides fragilis*, *Clostridium perfringens*, *Escherichia coli*, *Klebsiella pneumoniae*, or *Enterococcus faecalis* (60). In this study, biofilms formed by *C. albicans* provided a hypoxic microenvironment that could support the growth of the two strictly anaerobic bacteria, *B. fragilis* and *C. perfringens*—even though the biofilms were grown under oxygen-rich conditions. Thus, a *C. albicans* biofilm can act as a safe haven for anaerobic pathogens in oxygen-rich environments. Moreover, when cultured together with *C. albicans* in ambient oxygen, these anaerobes could induce the latter to form mini-biofilms, which, in turn, could protect the bacteria and allow them to proliferate under otherwise toxic conditions. Interactions occurring between different species in polymicrobial biofilms and their relevance to human health are clearly of great interest and importance.

PERSPECTIVES FOR DEVELOPING THERAPEUTICS FOR *C. ALBICANS* BIOFILM INFECTIONS

No biofilm-specific drugs exist today for *C. albicans* (or any microbe), making treatment of biofilm-based infections particularly problematic. However, a better understanding of the molecular mechanisms underlying biofilm formation and maintenance could lead to the development of new antifungals that specifically target the biofilm state. For example, identification of the molecular mechanisms behind biofilm dispersal could lead to a drug-based strategy that prevents it—or enhances it to the extent that a stable biofilm cannot form. A better understanding of adherence—both between *C. albicans* cells and a surface and between cells within the biofilm—could lead to strategies to prevent biofilm formation or to disrupt mature biofilms. Finally, a better understanding of the molecular basis of metabolic dormancy of subpopulations of cells, such as persister cells, might lead to strategies to reverse the physiology of these cells. These are only a few of the possible mechanism-based strategies that could be exploited to develop new biofilm-specific therapeutics.

Screens of chemical libraries have identified compounds that disrupt formation and/or maintenance of biofilms (25, 101, 185, 210). Although results from this approach are encouraging, it remains to be seen whether it will lead to useful therapeutics. Finally, any strategies that weaken *C. albicans* biofilm formation or maintenance could render biofilms susceptible to conventional antifungal drugs, making combination therapies effective, at least in principle. Current antifungal drugs have only two specific targets (ergosterol biosynthesis and 1,3-β-D-glucan synthesis), and it is widely believed that antifungal drugs with new mechanisms of action are needed (4, 18, 79). Our developing mechanistic knowledge of both the biofilm and the planktonic states (as well as the transition between the two) will hopefully be translated into new therapies to effectively clear biofilm-based infections and reduce relapse infection rates.

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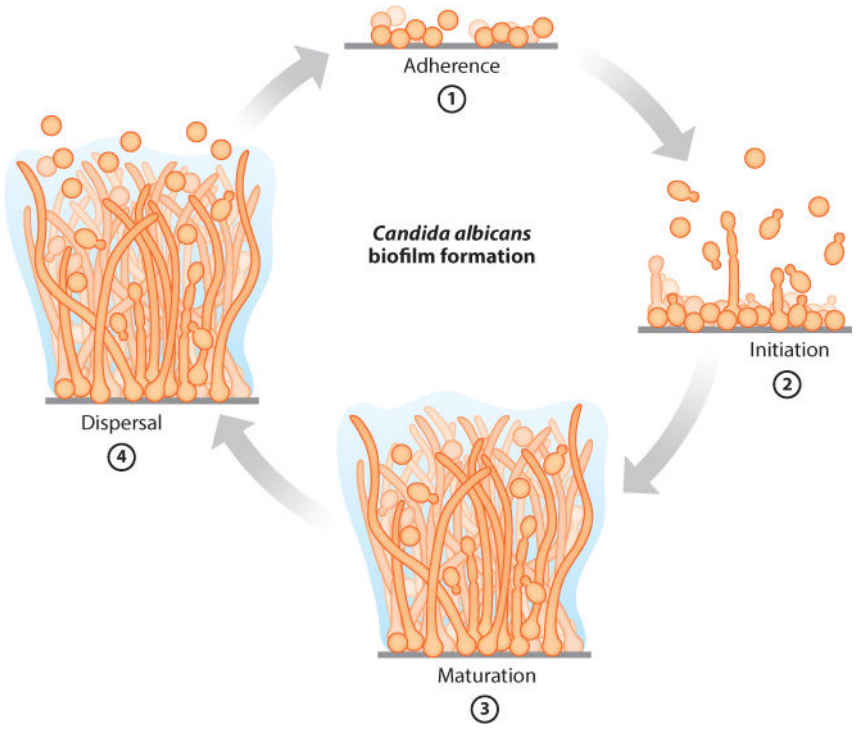

Figure 1.
Stages of *Candida albicans* biofilm formation. ① Adherence of yeast-form cells to a surface. ② Initiation of cell proliferation, forming a basal layer of anchoring cells. ③ Maturation, including growth of hyphae concomitant with the production of extracellular matrix material. ④Dispersal of yeast-form cells from the biofilm to seed new sites.

Table 1

C. albicans transcriptional regulators with roles in biofilm formation

ORF No.	Name	Biofilm phenotype[a]		Integrated in core network?[b]	Reference(s)
		In vitro	In vivo		
orf19.6124	*ACE2*	Defective	Defective	No	57, 86
orf19.2331	*ADA2*	Defective	NA	No	57
orf19.7381	*AHR1*	Defective	NA	Yes	8
orf19.4766	*ARG81*	Defective	NA	No	57
orf19.723	*BCR1*	Defective	Defective	Yes	140, 141
orf19.6874	*BPR1*	Defective	NA	Yes	59
orf19.4056	*BRG1*	Defective	Defective	Yes	140
orf19.4670	*CAS5*	Defective	NA	Yes	57
orf19.2356	*CRZ2*	No effect	Defective	Yes	57
orf19.3127	*CZF1*	Defective	NA	Yes	57
orf19.3252	*DAL81*	Defective	NA	No	57
orf19.610	*EFG1*	Defective	Defective	Yes	140, 163
orf19.3193	*FCR3*	Defective	NA	Yes	57
orf19.6680	*FGR27*	Defective	NA	No	57
orf19.1093	*FLO8*	Defective	Defective	Yes	59
orf19.5338	*GAL4*	Enhanced	Enhanced	Yes	59
orf19.1358	*GCN4*	Defective	NA	Yes	66
orf19.2842	*GZF3*	Defective	NA	Yes	59
orf19.4225	*LEU3*	Defective	NA	No	57
orf19.5312	*MET4*	Defective	NA	No	57
orf19.6309	*MSS11*	Defective	NA	Yes	193
orf19.2119	*NDT80*	Defective	Defective	Yes	140, 178
orf19.2012	*NOT3*	Defective	NA	No	57
orf19.7150	*NRG1*	Defective	NA	Yes	195
orf19.7247	*RIM101*	Defective	No effect	Yes	59

Annu Rev Microbiol. Author manuscript; available in PMC 2016 July 01.

ORF No.	Name	Biofilm phenotype[a]		Integrated in core network?[b]	Reference(s)
		In vitro	In vivo		
orf19.2823	*RFG1*	Defective	No effect	Yes	59
orf19.4590	*RFX2*	Enhanced	Enhanced	Yes	59
orf19.4662	*RLM1*	Defective	Defective	No	136
orf19.4998	*ROB1*	Defective	Defective	Yes	140
orf19.5871	*SNF5*	Defective	Defective	No	57
orf19.7319	*SUC1*	Defective	NA	No	57
orf19.798	*TAF14*	Defective	NA	No	57
orf19.5908	*TEC1*	Defective	Defective	Yes	140, 141
orf19.4062	*TRY2*	Defective	NA	No	57
orf19.1971	*TRY3*	Defective	NA	No	57
orf19.5975	*TRY4*	Defective	NA	Yes	57
orf19.3434	*TRY5*	Defective	NA	Yes	57
orf19.6824	*TRY6*	Defective	NA	Yes	57
orf19.4941	*TYE7*	Defective	NA	Yes	17
orf19.7317	*UGA33*	Defective	NA	No	57
orf19.1822	*UME6*	Defective	NA	Yes	194, 195
orf19.1035	*WAR1*	Defective	NA	No	57
orf19.3794	*ZAP1*	Enhanced	Enhanced	Yes	57, 63, 144
orf19.1718	*ZCF8*	Defective	NA	Yes	57
orf19.4767	*ZCF28*	No effect	Defective	No	57
orf19.5924	*ZCF31*	Defective	NA	Yes	57
orf19.6182	*ZCF34*	Defective	NA	No	57
orf19.7583	*ZCF39*	Defective	NA	No	57
orf19.6781	*ZFU2*	No effect	Defective	No	57
orf19.3187	*ZNC1*	Defective	NA	No	57

Abbreviations: NA, not available; ORF, open reading frame.

[a]Indicates biofilm-related phenotype of mutation, where mutation can cause a defect or an enhancement.

[b]Indicates that the gene is bound by any of the six “master” biofilm regulators (140).

Annu Rev Microbiol. Author manuscript; available in PMC 2016 July 01.

Table 2

C. albicans nonregulatory genes with roles in biofilm formation

Biofilm process	Genes	References
Adhesion	*ALS1*, *ALS2*, *ALS3*, *ALS5*, *EAP1*, *ECM33*, *HWP1*, *MP65*, *MSB2*, *PBR1*, *PGA1*, *PGA7*, *PGA10*, *PGA13*, *PGA26*, *PSA2*	65, 74, 102, 106, 138, 150, 155, 168, 170, 171, 177, 183, 213
Filamentation/cell wall	*AMS1*, *CAS4*, *CBK1*, *CHK1*, *CSA1*, *DSE1*, *GAL10*, *GAL102*, *GWT1*, *HGC1*, *HWP2*, *HYR1*, *KEM1*, *KIC1*, *MDS3*, *MED20*, *MFG1*, *MKC1*, *MOB2*, *NOT4*, *PMT1*, *PMT2*, *PMT4*, *PMT6*, *RBT1*, *RBT5*, *RHR2*, *SMI1*, *SPF1*, *SPT20*, *SUN41*, *SUR7*, *TPK1*, *TPK2*, *YAK1*	15, 37, 41, 49, 51, 55, 67, 68, 71, 93, 94, 98, 107, 136, 146, 149, 150, 165, 169, 177, 179, 182, 190, 196, 200, 208
Drug resistance	*CDR1*, *CDR2*, *MDR1*, *QDR1*, *QDR2*, *QDR3*	119, 126, 180
Extracellular matrix	*ADH1*, *ADH5*, *BGL2*, *CSH1*, *GCA1*, *GCA2*, *GSC1*, *IFD6*, *PHR1*, *XOG1*	7, 127, 144, 189
Dispersion	*HOS2*, *HSP90*, *PES1*, *SET3*, *SIF2*, *SNT1*, *YWP1*	70, 139, 167, 177, 194
Unknown	*AQY1*, *CAT2*, *GUP1*, *HSP21*, *HSP104*, *IPT1*, *NDH51*, *NUP85*, *OBPa*, *OBPalpha*, *ORF19.2175*, *ORF19.5412*, *PAP1*, *PAPalpha*, *PDX1*, *PIKa*, *PIKalpha*, *RIX7*, *SHA3*, *SOH1*, *SRB9*, *SSN3*, *SUV3*, *VAM3*, *VPS1*, *VPS4*, *YVC1*	14, 56, 58, 83, 103, 108, 109, 120, 146, 147, 165, 177, 184, 186, 188, 192, 196, 207

Table 3

Non–*C. albicans* biofilm formers in the Ascomycota phylum

Species	Reference(s)
Candida dubliniensis	73, 157, 187
Candida parapsilosis	73, 157
Candida tropicalis	73, 157
Candida krusei	73, 157
Candida glabrata	95, 157
Acremonium implicatum	211
Aspergillus fumigatus	125, 158, 176
Blastoschizomyces capitatus	16, 38, 39
Cladosporium sphaerospermum	211
Coccidioides immitis	40
Fusarium solani	211
Histoplasma capsulatum	154
Pneumocystis jirovecii	35, 36
Saccharomyces cerevisiae	26, 50, 128, 164
Scedosporium prolificans	30