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Highlights in pathogenic fungal biofilms

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A B S T R A C T

A wide variety of fungi have demonstrated the ability to colonize surfaces and form biofilms. Most studies on fungal biofilms have focused on \textit{Candida albicans} and more recently, several authors have reported the involvement of other genera of yeasts and \textit{Candida} species, as well as of filamentous fungi in the formation of biofilms, including: \textit{Cryptococcus neoformans}, \textit{Cryptococcus gattii}, \textit{Rhodotorula} species, \textit{Aspergillus fumigatus}, \textit{Malassezia pachydermatis}, \textit{Histoplasma capsulatum}, \textit{Paracoccidioides brasiliensis}, \textit{Pneumocystis} species, \textit{Coccidioides immitis}, \textit{Fusarium} species, \textit{Saccharomyces cerevisiae}, \textit{Trichosporon} \textit{asahii}, \textit{Mucorales} and \textit{Blastoschizomyces}. There is a current interest in describing the particular characteristics of the biofilm formation by of these fungi. A major concern is the control of biofilms, requiring knowledge of the biofilm mechanisms. However, our knowledge of these microbial communities is limited, due to the complexity of these systems and metabolic interactions that remain unknown. This mini-review aims to highlight recently discovered fungal biofilms and to compare them with the current knowledge on biofilms. This manuscript is part of the series of works presented at the “V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi” (Oaxaca, Mexico, 2012).

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Aspectos sobresalientes en la formación de biopelículas por hongos patógenos

R E S U M E N

Una amplia variedad de hongos poseen la capacidad para colonizar superficies y formar biopelículas (biofilms). La mayoría de los estudios efectuados sobre biopelículas de hongos han prestado atención a \textit{Candida albicans} y, más recientemente, varios autores han descrito la implicación de otros géneros de levaduras y especies de \textit{Candida}, al igual que de hongos filamentosos, en la formación de biopelículas, incluidos \textit{Cryptococcus neoformans}, \textit{Cryptococcus gattii}, especies de \textit{Rhodotorula}, \textit{Aspergillus fumigatus}, \textit{Malassezia pachydermatis}, \textit{Histoplasma capsulatum}, \textit{Paracoccidioides brasiliensis}, especies de \textit{Pneumocystis}, \textit{Coccidioides immitis}, especies de \textit{Fusarium}, \textit{Saccharomyces cerevisiae}, \textit{Trichosporon} \textit{asahii}, \textit{Mucorales} y \textit{Blastoschizomyces}. En la actualidad suscita interés la descripción de las características particulares de la formación de biopelículas de estos hongos. Una preocupación importante es el control de las biopelículas, que requiere una comprensión de los mecanismos de su formación. Sin embargo, nuestros conocimientos sobre estas comunidades microbianas son limitados debido a la complejidad de estos sistemas y a las interacciones metabólicas que aún no conocemos. Esta revisión tiene como objetivo poner de relieve las biopelículas fúngicas descubiertas recientemente y compararlas con los conocimientos actuales disponibles sobre ellas.

Este artículo forma parte de una serie de estudios presentados en el “V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi” (Oaxaca, México, 2012).

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It is estimated that 95% of the microorganisms found in nature are attached in biofilms. According to Costerton et al., a biofilm can be defined as a complex structured community of microorganisms, surrounded by an extracellular matrix of polysaccharides, adhered to each other at a surface or interface. This three-dimensional structure may become integrated naturally into any solid surface in contact with non-sterile water. Hence, these structures started to have great importance in diverse human activities. McCoy et al. were the first to describe the formation of biofilms in pipes. From this study, greater attention was given by researchers to this topic, after all the negative aspects of biofilm formation, and led the scientific community to seek alternatives to eliminate harmful biofilms that would cause damage to equipments through biocorrosion, product contamination, and represent significant losses to industries globally. If on one hand the biofilms can cause serious damage, on the other side they can be used in numerous bioprocesses. Examples include production of vinegar, pharmaceutical applications through the production of secondary metabolites, and biological processes for extracting metals from ores. Recognition of biofilms, from the 1980s on, contributed to recognize numerous persistent infectious diseases persistent as being caused by biofilms. Some infections caused by the use of medical devices in hospital environments such as catheters, are also related to biofilms. The extracellular polymers (EPS) matrix, which holds the biofilm cohesive, is also responsible for the persistence of biofilm-related infections, and protects microorganisms from disinfectants. Besides, resistance to UV radiation and dehydration (EPS matrix hydrated) has been demonstrated. This report aims to review the advances in fungal biofilms and in adhesins genes involved in biofilm formation, quorum sensing (QS), as well as to cover some new therapeutic strategies against fungal biofilms.

Fungal biofilms

Infections associated with the formation of biofilms are recognized as a significant and growing clinical problem; therefore, research in mycology has been increasingly focused on biofilm phenotyping. Recent advances in molecular techniques and confocal microscopy have shown that the formation of biofilms is the natural and preferred form of fungal growth and a major cause of persistent human infections. Microorganisms in biofilms grow in multicellular communities and produce an extracellular matrix that provides protection against from host defense mechanisms and antifungal drugs.

A wide variety of fungi have demonstrated the ability to colonize surfaces and form biofilms. Most studies on fungal biofilms have focused on Candida albicans and more recently, several authors have reported the involvement of other genera of yeasts and Candida species as well as of filamentous fungi in the formation of biofilms, including: Cryptococcus neoformans, Cryptococcus gattii, Rhodotorula species, Aspergillus fumigatus, Malassezia pachydermatis, Histoplasma capsulatum, Paracoccidioides brasiliensis (unpublished data), Pneumocystis species, Coccidioides immitis, Fusarium species, Saccharomyces cerevisiae, Trichosporon asahii, Mucorales, and Blastoclizomyces. There is growing interest in uncovering the true participation of fungal biofilms in human disease. These formations play an important role in the development of infections, since microorganisms that grow in biofilms exhibit unique phenotypic characteristics when compared to their planktonic counterparts. These characteristics include increased resistance to host defense mechanisms and antibiotic therapy.

The adherence of a biofilm to the host may trigger an acute fungemia and/or disseminated infection. This occurs when cell clusters are dispersed from the initial biofilm and occupy a niche not previously colonized. A recent study developed by Updurai et al. demonstrated that cells that detach from a biofilm have a greater association with mortality as compared to planktonic microorganisms. In fact, over 65% of human infections involve the formation of biofilms, which is related to the increasing use of biomaterials in medical practice and the increasing number of immunocompromised patients. In addition, more than 500,000 deaths per year are caused by biofilm-associated infections.

As a result, biofilms have important and, often, deleterious effects on human health. Fungal biofilm formation on catheters and prostheses contributes to the development of nosocomial infections. According to Kojic et al., the persistence of fungal infections occurs due to the ability of a fungus to form biofilms on a wide variety of medical devices and because of persisting cells representing an important mechanism of resistance. Once infected, the in vivo eradication of a biofilm usually requires the administration of toxic concentrations of antimicrobials, and the recommended treatment includes removal of the contaminated device; however, this is a difficult and costly procedure that can result in medical complications. Therefore, fungal biofilms have become a major clinical and economic problem.

Multidrug tolerance is caused by a small subpopulation of microbial cells termed persisters that become a reservoir from which recurrence of infection may be developed. These cells are responsible for an important mechanism of resistance in chronic infections extensively studied in bacteria, which have attracted some attention recently in the context of fungal biofilms. In C. albicans biofilms, a small subset of yeast cells have been described that is highly resistant to amphotericin B, following adhesion, and this is independent of the upregulation of efflux pumps and cell membrane composition. C. albicans persisters were detected only in biofilms and not in diverse planktonic populations. When a biofilm was killed with amphotericin B and reinoculated with cells that survived, a new biofilm was produced with a new subpopulation of persisters; this suggests that these cells were not mutants but phenotypic variants of the wild type. The basis of this drug resistance is not clear and involves different mechanisms, including the presence of a small number of persisters, which are cells that survive high doses of an antimicrobial agent. Unlike bacterial persisters, C. albicans persisters have so far been observed only in biofilms and not in planktonic populations. Identification of important cellular components that are responsible for the occurrence of persisters in fungal biofilms could open the way to the rational design of antibiofilm agents.

Recent findings have reported the involvement of new fungal genera and species in the formation of pathogenic biofilms and it is important to look for the role they can play in infections. There is a current interest in describing the particular characteristics of biofilm formation of the species Rhodotorula, A. fumigatus, M. pachydermatis and the dimorphic fungi H. capsulatum, Coccidioides spp., and Paracoccidioides spp. It was also recently demonstrated that Rhodotorula species are able to form biofilms. The increase in invasive infections caused by emerging pathogens such as Rhodotorula is related to the increased occurrence of degenerative and malignant diseases in different populations, the growing number of patients who undergo organ transplantation therapies that include immunosuppression, broad-spectrum antibiotics and invasive medical procedures; and the use of implantable medical devices, such as central venous catheters, which facilitate the formation of biofilms by these pathogens, causing fungemia followed by eye infections, peritonitis, and meningitis. Nunes et al. studied various isolates of Rhodotorula species and noted that this genus is able to form biofilms, which could play a role in the pathogenesis...
of infections caused by these species. Canabarro et al. isolated *Rhodotorula* sp. in association with *C. albicans* subgingival biofilms from patients with severe chronic periodontitis.

Recent reports describe the growth of biofilm structures for the filamentous fungus *A. fumigatus*. This species is responsible for approximately 90% of cases of invasive aspergillosis, a severe infectious disease characterized by high mortality rates. Aspergillus colonization and biofilm formation predominantly occurs in patients with genetic functional lung abnormalities, such as cystic fibrosis or chronic obstructive pulmonary disease. Biofilms of *Aspergillus* can affect diverse biomaterials, such as catheters, prostheses, cardiac pacemakers, heart valves and breast implants. In addition, a spherical mass of hyphae, called aspergilloma, can form in the respiratory tract or the urinary tract. All clinical antifungal drugs are significantly less effective under the biofilm or spherical hyphae conditions, suggesting that there is need for high dosages or antifungal combination therapy for better penetration of drugs in biofilms.

Another pathogen that has received growing attention is the fungus *M. pachydermatis*, capable of forming in vitro biofilms on devices commonly used in the medical practice, including polystyrene microplates and polyurethane catheters. *M. pachydermatis* is a commensal yeast found on the skin and mucosa of healthy dogs and cats, but has become an important pathogen of human fungemia in intensive care units and has been isolated from preterm neonates, children and adults. These infections are directly associated with the formation of biofilms on catheters in patients receiving parenteral nutrition with lipid formulations.

Recently, an in vitro study demonstrated the efficiency of *H. capsulatum* to form biofilms on abiotic surfaces. *H. capsulatum* is the causative agent of histoplasmosis, a systemic fungal disease that has become a major health problem in Latin America and worldwide. High concentrations of this fungus are found in areas with bird and bat droppings, such as caves, chicken coops, or even urban buildings. A study by Pitanguy et al. determined the pattern of infection of *H. capsulatum* in epithelial cells, characterized as a compact mass of yeast cells, which possibly leads to the formation of a complex three-dimensional architecture of biofilms and promotes the internalization of yeast into host cells. A previous study by Suarez-Alvarez et al. described the profile of *H. capsulatum* yeast adhesion on different bat organs. That study also noted that the yeasts are found in clusters in the lung parenchyma, spleen, liver, and intestine. Recent advances in high-throughput methods for the investigation of biofilms opened the possibility of starting an “omics” approach to study these complex structures in the next decade. Additionally, in vivo studies are needed to define the true role and growth regulation of *H. capsulatum* biofilms.

Paracoccidioidomycosis is a systemic mycosis of great relevance in Latin America, especially in Brazil, which has the highest concentration of endemic areas, as more than 80% of the reported cases occurred in this country. The causative agents are the dimorphic fungi *P. brasiliensis* and *P. lutzii*. These fungi have several virulence factors that can cause harm to the host. The adhesion, colonization and characteristics of these fungi enable them to withstand the hostile environments of the host and are correlated with the development of the disease. Adhesion is a widely distributed phenomenon that is shared by many microorganisms, enabling them to colonize in their habitats. Many fungi, especially pathogenic fungi, are able to adhere to host tissues, which is the first step in the process of biofilm formation. The present authors were able to demonstrate biofilm formation by *P. brasiliensis*. Those experiments were performed in vitro, with the fungus forming biofilms at low oxygen tensions (unpublished data).

Davis et al. described recurrent coccidioidal meningitis and *C. immitis* biofilm was found on the tip of the ventricle-peritoneal shunt tubing despite the patient’s taking an adequate dosage of fluconazole.

### Quorum sensing in fungal biofilms

The regulation of the expression of virulence genes is a crucial step in pathogenesis and in microorganism adaptation to host tissues. QS is a mechanism of microbial communication dependent on cell density that can regulate several behaviors in bacteria such as secretion of virulence factors, biofilm formation, competence and bioluminescence. It is a major mechanism of microbial communication and QS occurs by the continuous release and monitoring of hormone-like molecules called auto-inducers or QS molecules.

QS has been observed in many bacterial species regulating the most diverse processes, including secretion of virulence factors, biofilm formation, and antibiotic production; now, it is believed that the same occurs in fungi. In pathogenic microbes, the coordinated expression of virulence factors during infection of a host probably constitutes a significant survival advantage by enhancing the chances of establishing infection and escaping the immune response. Several molecules have been described as belonging to QS. Lipids, such as sphingolipids, farnesol and oxylipins are signaling molecules in pathogenic fungi. Recently, aromatic alcohols phenylethanol and tryptophol molecules were identified as quorum-sensing in *S. cerevisiae*. These compounds, which are also produced by *C. albicans*, showed growth on *S. cerevisiae* pseudohyphae at relatively low concentrations.

Farnesol and tyrosol are QS molecules in *C. albicans*. The primary mechanism of regulation of QS is the production of auto-inducers that are released into the external environment, where they accumulate and concomitant measurement of their concentration is achieved through its interaction with its receptor, which may be as much as being in intracellular cell surface. In bacteria, these inducers have been widely studied and they are related to various cellular processes, such as antibiotic production, sporulation ability, and expression of virulence genes, DNA transfer and formation of biofilms. Shirrell et al. have shown that 40 mM or 100 mM farnesol concentrations are able to induce high regulation of *C. albicans* protein involved in protection against oxidative stress. Sharma et al. demonstrated that farnesol can modulate the action of drugs on planktonic cells of *C. albicans*. Ramage et al. evaluated the effects of farnesol on biofilm development and observed that farnesol inhibits the formation of hyphae when added in the initial phase of biofilm formation and, hence, can compromise the structure. Other studies have shown detrimental effects of farnesol on many microorganisms, including fungi and bacteria, such as *Staphylococcus aureus*, *S. cerevisiae*, *Aspergillus* species, *P. brasiliensis* and *Mycobacterium smegmatis*. The high density of microorganisms in biofilms led to speculate that detection of QS plays a specific and important role in the physiology of biofilms. In other bacteria, QS detection can function in the dispersion of individual organisms from biofilm. It seems, therefore, that the morphogenesis in *C. albicans* is under control of antagonistic tyrosol and farnesol. Both farnesol and tyrosol in biofilms have been studied to emphasize the morphological aspect. The same can happen with other fungi, potentially increasing the efficacy of drugs, leading to new strategies for the treatment of fungal infections.

### Genes involved in the formation of fungal biofilms

It is clear that the current knowledge on fungal biofilms has increased significantly and much of that knowledge has been gained through in vitro and in vivo studies of *Candida* biofilms. Through research focused on the biofilm of *C. 
*Candida albicans*, the molecular characteristics of fungal biofilm development were elucidated. Other studies demonstrated that the increase of microorganisms is associated with infections of several medical devices, leading to high mortality (approaching 40%).

Recently, the transcriptional network that governs the development of biofilms for *C. albicans* was identified. This network consists of six master transcription regulators (EFG1, TEC1, BCR1, NDT80, ROB1, and BRG1) and approximately 1000 target genes, whose expression is controlled by these regulators. The six master regulators were identified by screening a library of approximately 165 mutant transcripts during in vitro biofilm formation and observing that the mutants had changed during the event. Six deletion mutants that produced defects in biofilm formation were identified, three are new (ROB1, BRG1 and NDT80) and three were previously known to play a role in the development of biofilms (BCR1, TEC1 and EFG1). All six identified genes were associated with defects in both in vitro and in vivo biofilm formation.

Banerjee et al. studied the role of UME6 and found it to be a regulator of hyphae in *C. albicans* biofilms. Another study, performed by Tafl et al., demonstrated that three enzymes were related to the production of extracellular polysaccharides, encoded by genes BGL2, PHR1, and XOG1. It has been shown that these enzymes are essential for the delivery of β-1,3-glucan for the matrix of the biofilm biomass and accumulation of a mature, extracellular matrix. Through the construction of mutants, researchers have demonstrated an increased biofilm susceptibility to commonly used antifungals, such as fluconazole. These investigators have proposed that the discovery of inhibitors of these enzymes provide promising anti-biofilm effects. The use of molecular biology tools has helped to unravel the “mystery” of microbial biofilms. Much has been discovered; however, despite advances in technology and arrays to evaluate enzymes and proteins, a way to completely eliminate biofilms has yet to be discovered.

### Adhesins in fungal biofilm

Adherence is a precondition for colonization and an essential step in the establishment of infection. Adherence is mediated through a large number of differentially regulated cell wall–bound adhesins. Studies with *Candida* spp. and *P. brasiliensis* have shown that these fungi have great potential for adherence to epithelial cells.

Among several groups of genes involved in biofilm formation, it was found that the family ALS (agglutinin-like sequence), present in *C. albicans*, *Candida tropicalis* and *Candida glabrata*, plays a key role in this process and encodes proteins having the characteristics of adhesin glycoproteins on the cell surface. It has been shown that ALS genes exhibit increased expression during the formation of biofilm. The family present in *C. albicans* ALS includes eight genes (ALS1–ALS7 and ALS9) encoding many surface glycoproteins. Molecular studies on the expression of ALS genes showed that they are differentially expressed and regulated as a function of cell physiological processes, such as the growth stage and cell morphology, i.e., yeast or predominantly in the form of hypha and pseudo-hypha. ALS1, encoding cell surface glycoproteins, exhibits high expression in *C. albicans* biofilm cells. Gene ALS3 also showed high expression, however, it is apparently associated with the production of *C. albicans* bypae. Nails et al. compared gene expression of ALS1 and ALS3 among cells of *C. albicans* biofilm formed on the surface of silicone and on suspended cells (planktonic) and found a significant increase in the expression of ALS1 biofilm cells, and decreased expression of ALS3. Moreover, Nobile et al. concluded, after several tests with mutants als1/als1 als3/als3 that ALS3 and ALS1 are essential for biofilm formation in vivo and reduced expression of these proteins entails the formation of a fragile biofilm, whereas their functions are compatible with biofilm structure and biochemical property. Zhao et al. demonstrated that the decrease in ALS2 protein expression resulted in the reduction of biofilm biomass, suggesting that ALS2 contributes to the later stages of biofilm development and not to the adherence stage. In an experimental model of catheter infection in vivo, ALS1 and ALS3 also had redundant functions, and other highly expressed genes of the family – ALS5, ALS6, ALS7, and ALS9 – were able to partially or completely replace the absence of ALS1 and/or ALS3, facilitating the development of biofilm in such an experimental model, whereas ALS2 and ALS4 were unable to do so, and all ALS genes could be replaced by ALS3 or ALS1 models in vivo and in vitro.

*C. albicans* adheres to epithelial cells in culture, mainly through EAP1 adhesion. EAP1 is a member of a family of up to 23 putative adhesin–encoding genes present in this yeast genome. EAP1 expression in vitro is controlled both positively and negatively; in addition, it presents high cell–to–cell heterogeneity, which depends on Sir–mediated silencing. EPA6 also encode functional adhesions in *C. glabrata*.

Some molecules of *H. capsulatum* have been identified as ligands of extracellular matrix components. McMahon et al. reported that a 50-kDa protein present in the fungus cell walls is able to bind to laminin, an extracellular matrix component of host lung cells. This protein is an essential step in the pathogenesis of the fungus, once in the alveolar macrophages yeasts inhibit the production of proinflammatory cytokines, facilitating infection. There are no studies demonstrating *H. capsulatum* adhesins related to biofilm formation.

Pathogenic fungi such as *Paracoccidioides* spp., have multiple factors that can cause damage to the host and contribute to the virulence phenotype. Adhesion, colonization and characteristics of fungi enable them to resist the hostile environments of the host and are correlated to disease development. Further, this protein has virulence potential with high affinity for laminin, thereby increasing the capacity of the fungi to invade and destroy tissues. Adherence of *Paracoccidioides* to epithelial cells is also greatly reduced in the presence of anti-gP43. Gp43 also interacts with fibronectin, another component of the extracellular matrix. Other adhesion molecules in *P. brasiliensis* have also been described, such as a 30-kDa adhesion molecule, with the ability to bind to laminin, and are expressed in *P. brasiliensis*, isolates with high adhesion capacity. Enolase is a cytoplasmic enzyme most abundantly expressed in many microorganisms. Thus, for many years enolase was seen as a soluble glycolytic enzyme, present exclusively in the cytoplasm. In 2009, Donofrio et al. demonstrated that enolase from *P. brasiliensis* (PbEno) is a fibronectin-binding protein and genetic and proteomic evidences support its localization on the cell surface. Studies conducted in the Clinical Myology Laboratory, Unesp, Araquara, Brazil, have shown an increase of some adhesins of *P. brasiliensis* in hypoxic conditions, precisely the condition that occurs in biofilm formation (unpublished data).

### Antibiofilm strategies

Fungal biofilm resistance mechanisms include extracellular matrix, efflux pump activity, persisters, cell density, overexpression of drug targets, stress responses, and the general physiology of the cell. Thus, to increase the efficiency of new treatment strategies against bacterial and fungal infections, factors that lead to biofilm growth inhibition, biofilm disruption, or biofilm eradication are being sought. These factors could include enzymes, sodium salts, metal nanoparticles, antibiotics, acids, chitosan derivatives, or plant extracts. Biofilm formation almost always leads to a large increase in resistance to antimicrobial agents (up to 1000-fold decrease in susceptibility) in comparison with planktonic cultures.
grown in conventional liquid media. Many studies have focused on the search for natural or synthetic products for various fungal biofilms, but biofilms of Candida species are the most studied.

Studies performed by Pires et al. showed the presence of biofilms in the fluid pathways of hemodialysis machines. The impacts of four biocides used for the disinfection of hemodialysis systems were tested against Candida parapsilosis and Candida orthopsilosis biofilms generated by isolates obtained from a hydraulic circuit, and collected in a hemodialysis unit. Acetic acid was shown to be the most effective agent against Candida biofilms. Strategies for effective disinfection procedures used for hemodialysis systems should also seek to kill and inhibit biofilms. On the other hand, some natural products have been tested against C. orthopsilosis and C. parapsilosis on planktonic and biofilm conditions and could be natural anticandidal agents that can be effectively utilized for the control of the yeasts. In the Clinical Mycology Laboratory, UNESP, has been consolidating a platform for the development of antifungal and bioreagents. This platform is based on FAPESP programs, such as the Biota-FAPESP, the BIOPROSPECTA, and also in SISBOTA – CNPq. Among natural substances evaluated that deserves highlighting lies maytenin with antifungal potential against several fungal species.

Another promising strategy is the antifungal activity of silver nanoparticles. Silver (Ag) has been well known for its antimicrobial characteristics, and has a long history of applications in medicine with a well-tolerated tissue response. In the hope of inhibiting biofilm formation, thereby reducing the chance of microbial infections and rejection, AgNP has been used for lining of medical implants with titanium. Recently, Sun et al. reported the antibiofilm activity of terpinen-4-ol loaded lipid nanoparticles against C. albicans biofilms and this compound (10 μg/ml) eradicated formed biofilms.

Studies with antibodies have been performed by several authors to test their effects on diverse fungal and bacterial organisms. The latest therapeutic treatment of Cryptococcus biofilms suggests that monoclonal antibodies (MAbs) are potentially useful in clinical treatment. Martinez et al. demonstrated that alpha radiation, guided by MAb, effectively impairs fungal biofilm formation. Other authors have found that administering a prophylactic dose of antibodies specific to biofilms, immediately after insertion of a medical device, is effective in managing biofilm formation.

Another important therapeutically promising is photodynamic therapy (PDT), widely used for species of Candida biofilms. Several authors have associated light emitting diode with other substances. There are two major types of cellular damage: DNA damage and the destruction of cellular membranes and organelles. Recent studies have shown that the antimicrobial effect can be obtained with the use of photosensitizers belonging to different chemical groups. Junqueira et al. assessed the PDT on biofilms of Candida spp., Trichosporon mucoides, and Kodamaea ohmeri.

Because the biofilm matrix is composed of DNA, proteins, and extracellular polysaccharides, recent studies have indicated that the disruption of the biofilm structure could be achieved via degradation of individual biofilm compounds by several enzymes such as DNase, lactonases, α-amylases, and lyase.

Research methodology used recently in biofilms

Infections associated with biofilm formation are resistant to conventional antifungal therapy and due to the high morbidity and mortality caused by these formations there is an urgent need to use new technologies and innovative therapies for success in eradicating these infections. In this sense, the ease of working with new models, in vivo approaches of "omics" techniques of molecular biology and nano science are innovative avenues of research that have paved the way for new lines of study in the search for antifungal targets.

In vitro biofilm models are needed to elucidate mechanisms of development of biofilms. Nevertheless, results of testing in vitro of biofilm formation by clinical isolates do not always agree with results in vivo. In this context, invertebrate models become useful to visualize infection, determining the true role of biofilms in infectious processes and how these formations directly affect the health of the host. A recent review published by Edwards and Kjellerman highlights the advances in cell–cell interactions and the understanding how host immune system reacts to biofilm formation in five invertebrate models: Lemna minor (duckweed), Arabidopsis thaliana (thale cress), Dictyostelium discoideum (slime mold), Drosophila melanogaster (common fruit fly), and Caenorhabditis elegans (roundworm). These models were described and assessed for their relevance to infections associated with polymicrobial biofilm formation. According to the authors, it is possible to use each of these models to investigate the peculiar characteristics of such biofilm, however C. elegans is presented as the most complete model to elucidate virulence factors, host innate immune function and to visualize the infection. Some authors have adopted C. elegans as a model to determine the toxicity and antifungal activity of fungicidal compounds, aimed at the discovery of new targets for the treatment of biofilms of C. albicans. Thus, the nematode C. elegans has given rise to promising perspectives for innovative human therapies.

Concomitantly, another important branch of research should be stressed, “omics” approaches have been widely exploited by pharmaceutical and biotechnology companies for the development of safer and more effective drugs. Currently, there is great interest in the search for effective drugs against novel targets and, in this context, we highlight proteins by proteomic analysis, since the identification of a target protein essential to cell survival can provide important information for the treatment of mycoses. The proteome of C. albicans in planktonic and biofilm cultures is well documented by several authors. In this sense, our group in Brazil has noted that there is a different pattern of proteins when comparing H. capsulatum in planktonic and biofilm cultures. Using mass spectrometry more than 40 proteins, belonging to different functional groups, were differentially expressed and identified between the biofilm and dispersed cells, and the three main functional groups include proteins involved in the metabolism of amino acids, nuclear proteins, and translation protein (unpublished data). Additionally, our group has been working on the standardization of methodologies that aim to characterize the differential transcriptional profile exhibited by fungi shaped biofilm and planktonic conditions through transcriptomics analysis. In addition, we aim at identifying the secreted molecules and metabolites, generated during biofilm formation, using secretomic and metabolomics analyses, respectively. These techniques should allow targeting cellular receptors for biofilm disruption in the interaction with host cells.

Conclusions

Biofilms control is necessary and has been the subject of many investigations in the fields of biotechnology and public health, as biofilms are present in many situations, from human disease to industry. A major concern is the control of biofilms, for which knowledge of biofilm mechanisms is essential. However, information of microbial communities is sparse, due to the complexity of these systems and to metabolic interactions that remain unknown. For this reason, advances in high-throughput methods have allowed the interaction of systems, combining genomics, transcriptomics, proteomics, and metabolomics to elucidate the real function and ecology of these complex formations.


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Ramos G, Mowat E, Jones S, Williams C, Ramage G. Development of a simple model for studying the effects of antifungal agents on multicellular communi-


