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Highlights in pathogenic fungal biofilms

Janaina De Cássia Orlandi Sardi[a,c], Nayla De Souza Pitangui[a,c], Gabriela Rodríguez-Arellanes[b], Maria Lucia Taylor[b], Ana Maria Fusco-Almeida[a,d], Maria José Soares Mendes-Giannini[a,*,d]

[a] Department of Clinical Analysis, Laboratory of Clinical Mycology, Faculty of Pharmaceutical Sciences, Universidade Estadual Paulista (UNESP), Araraquara, São Paulo, Brazil
[b] Fungal Immunology Laboratory, Department of Microbiology and Parasitology, School of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico

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ABSTRACT

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*, *Pneumocystis* species, *Coccidioides immitis*, *Fusarium* species, *Saccharomyces cerevisiae*, *Trichosporon asahii*, Mucorales and *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).

Aspectos sobresalientes en la formación de biopelículas por hongos patógenos

Palabras clave:
Biopelículas
Quorum sensing
Patógenos fúngicos
Adhesinas
Tratamiento anti-biopelículas

RESUMEN

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 *Candida albicans* y, más recientemente, varios autores han descrito la implicación de otros géneros de levaduras y especies de *Candida*, al igual que de hongos filamentosos, en la formación de biopelículas, incluidos *Cryptococcus neoformans*, *Cryptococcus gattii*, especies de *Rhodotorula*, *Aspergillus fumigatus*, *Malassezia pachydermatis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, especies de *Pneumocystis*, *Coccidioides immitis*, especies de *Fusarium*, *Saccharomyces cerevisiae*, *Trichosporon asahii*, mucorales y *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).

* Corresponding author.
E-mail address: gianninimj@gmail.com (M.J.S. Mendes-Giannini).
[c] Equal contribution to the development of the review.
[d] Supervisors.

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It is estimated that 95% of the microorganisms found in nature are attached in biofilms. According to Costerton et al.,[21] 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.[139] Hence, these structures started to have great importance in diverse human activities. McCoy et al.[80] 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,[59] 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,[10] citric acid,[114] pharmaceutical applications through the production of secondary metabolites,[96] and biological processes for extracting metals from ores.[109] Recognition of biofilms, from the 1980s on, contributed to recognize numerous persistent infectious diseases persistent as being caused by biofilms.[22] Some infections caused by the use of medical devices in hospital environments such as catheters, are also related to biofilms.[32] The extracellular polymers (EPS) matrix, which holds the biofilm cohesive, is also responsible for the persistence of biofilm-related infections,[20] and protects microorganisms from disinfectants. Besides, resistance to UV radiation and dehydration (EPS matrix hydrated) has been demonstrated.[14,139] 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 in biofilm phenotyping.[57] 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.[22]

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 *Blastoschizomyces*.[13,25,26,28,31,75,88,99,104,110,122,137]

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.[104] These characteristics include increased resistance to host defense mechanisms and antibiotic therapy.[78]

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.[106] A recent study developed by Uppuluri et al.[133] 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.[19,107] In addition, more than 500,000 deaths per year are caused by biofilm-associated infections.[89]

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.[135] According to Kojic et al.,[63] 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.[115] 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.[43] 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,[7,106,115] which have attracted some attention recently in the context of fungal biofilms.[9] 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.[65] 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.[68,115]

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.[37,89,99,105,106]

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[131]; 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.[29,116,131,132] Nunes et al.[94] studied various isolates of *Rhodotorula* species and noted that this genus is able to form biofilms, which could play a role in the pathogenesis