**Candida albicans** biofilm: formation and antifungal agents resistance

Tri Wibawa  
Department of Microbiology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

**ABSTRACT**

*Candida sp* are the most common fungal pathogens causing fatal health care associated infections. Among the genus of Candida, *Candida albicans* is the most frequent species isolated from patients. The notorious *C. albicans* infection is the ability of this dimorphic fungus to form biofilm. Biofilm has been pointed as a dynamic phenotypic switching in bacteria and fungi, which may result in higher morbidity and mortality in human beings. This review addresses the basic explanation of biofilm formation which is characterized by the antifungal agents resistance. The factors that influence *C. albicans* biofilm formation and antifungal agents resistance are discussed.

**INTRODUCTION**

*Candida sp* are documented as a causative agent of plenty fungal infection in human beings. *Candida sp* are the most common fungal pathogens causing fatal health care associated infections, especially in patients admitted to intensive care units and the fourth most frequent pathogen isolated and accounted for 9% of blood stream infection. Colonization and biofilm formation of *Candida sp* has been reported in the biomaterials, such as: shunts, stents, prostheses, implants, catheters, and other indwelling medical devices. The *Candida sp* isolated from patients suffering from nosocomial blood stream infection in the Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) study in the United States were *Candida albicans* (54%), followed by *C. glabrata* (19%), *C. parapsilosis* (11%), and *C. tropicalis* (11%) respectively. *Candida albicans* has been reported as a common causative agent for
nosocomial infection transmitted among patients in burn units, and was documented as the most common cause of recurrent and non-recurrent vulvovaginitis.

*Candida albicans* is a yeast form fungi which is well known as a classic example of opportunistic fungi which may be responsible for various superficial and systemic infection in human beings. *Candida albicans* is frequently found as the normal microbiota of humans and does not regularly cause disease in immunocompetent hosts. It exists as commensal in the skin and mucosal surfaces, as well as genital and gastrointestinal tracts. However, in immunocompromised patients it may be responsible for mild to severe clinical manifestation.

*Candida albicans* becomes pathogen if the host’s immune response is impaired. The impaired local or systemic immune response may contribute to the alteration of commensal-pathogen characteristic of *C. albicans*. Neutropenia, neutrophil dysfunction, disruption of mucosal barriers, patients receiving chemotherapy for neoplasm, and immuno-suppressants after organ transplantation or patients with HIV/AIDS are the risk factors for disseminated opportunistic fungal infections. In an immunocompromised host, translocation from the gastrointestinal tract and intravascular catheters are the two main sites of entry for disseminated candida infection. In HIV/AIDS patients, *C. albicans* was observed as the leading fungi that causes opportunistic infection.

*Candida albicans* is unicellular, reproduced by budding, and grows well in routine automated blood culture bottles and on agar plates. *Candida albicans* is dimorphic fungi that is characterized by its ability to grow alternately as yeast and filamentous forms fungi. In specific conditions it can be found in where most cells grow as hyphae or filamentous form. The morphological change of *C. albicans* is rapid and in response to the external signals. These dynamic changes are associated with the pathogenicity and virulence of the microorganism.

Other than dimorphic (yeast-hyphae) biological property, *C. albicans* has morphological characteristics which may occur naturally in its life cycle. These distinct morphologies include the pseudohyphal form, opaque form and chlamydospore. Pseudohyphae is related with its budding reproduction property. It is often found with yeast and hyphal forms in vegetative culture and during infection. It is well known that *C. albicans* may produce pseudohyphae and true hyphae at the same time (FIGURE 1). The opaque form is associated with mating-competent cells. The white-opaque transition is associated to the sexual mating process in *C. albicans*.

![FIGURE 1. Microscopic appearance of Gram staining of sputum obtained from HIV/AIDS patient that showed colonization of *C. albicans*. The budding yeast, true hyphae and pseudohyphae were observed.](image-url)
useful to modify the deleterious impact of antifungal resistant.

**DISCUSSION**

**Biofilm formation of *C. albicans***

Biofilm can be defined as a community of microorganisms that are irreversibly attached to a surface, containing exopolymeric matrix and exhibiting distinctive phenotypic properties. The most important distinctive phenotypic property of biofilm forming microorganism is the antimicrobial resistance. This might result to a critical situation in patient setting, as in some circumstances it means the necessity of removing the prosthesis, implant or other medical device. The increases of morbidity and mortality as well as medical expenses are the inevitable consequences.

*Candida albicans* is not the only species included in the Candida genus which is able to develop biofilm. Indeed, *C. albicans* biofilm is the most studied biofilm formed by yeasts. Three stages of *C. albicans* biofilm formation are hypothesized as: the adherence of yeast cells to the surface (initial phase), formation of an extracellular matrix with dimorphic switching from yeast to hyphal forms (intermediate phase), and increase in the extracellular matrix material recruitment to form three dimensional structure of biofilm (maturation phase) (FIGURE 2).\(^3,14\) It begins with adherence of yeast cells to a foreign substrate (host tissue or medical device) (A), followed by proliferation of the yeast cells across the substrate surface with hyphal development which may include the pseudohyphae and true hyphae (B). The final step of biofilm development is the maturation phase with recruitment of massive extracellular matrix (brown) from the environmental substrate that may produced by the host or the microorganisms that contribute to the biofilm development (C). After entering maturation phase, a few planktonic cells may be released from the mature biofilm and transferred to the new surface to start the new biofilm formation cycle.

![FIGURE 2. Cartoon of step-wise process of *C. albicans* biofilm development](image)

In every step of biofilm formation, there are dynamic gene expressions that may facilitate the biofilm formation. Some genes are specifically expressed in planctonics, initial phase, intermediate phase or maturation phase.\(^15\) There are plenty genes that have been identified involved in cell adhesion (42 genes), biofilm formation (122 genes), filamentous growth (512 genes), and phenotypic switching (44 genes) respectively.\(^15\) Very recently, Nobile *et al*\(^17\) described a master circuit of six transcription regulators, sequence-specific DNA-binding proteins that regulate transcription, which controls biofilm formation by *C. albicans*. Exploring the regulation of genes expression in the biofilm formation phases is important to
understand the characteristic of candidal biofilm. It is even more important to find the molecular target to inhibit the biofilm formation.

The initial phase is started when C. albicans cells attached to the surface of living or unanimated material such as, mucosal layer, dental surface, or indwelling medical devices. It is important to consider that cells-surface adherence of the yeast is the key point of biofilm development. It needs to anchor the structure on the surface. On the other hand, the adherence among yeast cells itself has been also highlighted as key point of initial phase of biofilm formation properties. The adherence of yeast cells to the surface were regulated by the environmental milieu. For example in oral cavity, the adherence was influenced by the dietary and salivary factors. The presence of two monosaccharides, glucose and galactose, has been extensively investigated for their effects on candidal adhesion. This finding was in agreement with another report which compared the biofilm formation in vitro by using two difference media, RPMI and synthetic urine. The level of hyphal formation of yeast cells biofilms formed in synthetic urine medium was diminished compared to those grown in RPMI medium.

The nature of medical devices surface is important to build a candida biofilm. The property of medical devices surface, contact angle of materials, and the index of hydrophobicity were found to be correlated positively with initial adhesion and biofilm formation of C. albicans. Modification of medical devices surface has been showed as candidate approach to inhibit candida biofilm formation. The surface support for biofilm formation is depending on the nature of biomaterial. It was showed in the C. albicans biofilm formation model that latex and silicone elastomer increased the biofilm formation but not the polyurethane or pure silicone. Nonetheless, there was a negative correlation between mucin absorption with removability of candida biofilm.

Intermediate phase was characterized by formation of an extracellular matrix with dimorphic switching from yeast to hyphal forms. However, switching of the yeast to hyphal form was not obligated for C. albicans biofilm. Two mutants of C. albicans derived from one parental strain were characterized as incapable to form hyphae or yeast. Experimental procedures using these two mutants showed that it still showed the ability to form biofilm. It was observed that the characteristic of the biofilms of the two mutants was different. The hypha-negative mutant produced only the basal layer, and the yeast-negative mutant produced only the outer layer, which was more easily detached from the catheter disks. This suggests that dimorphism might be necessary for biofilm architecture and structure. This result is in agreement with the report that found farnesol, a quorum-sensing molecule, inhibit filamentation in C. albicans, also inhibits its biofilm formation. However, it should be noted that biofilm somehow absolutely does not depend to the morphological properties of the fungi. Either yeasts or hyphal morphology may contribute to the potential effect of biofilm formation.

Important to the maturation of candidal biofilm is recruitment and deposition of extracellular matrix. This complex extracellular material might function to defend against phagocytic cells of host immune response, to serve as a scaffold to maintain biofilm structure integrity, and to safe guard the biofilm from environmental exposure. Environmental factor that may be important to the candida biofilm is the bacterial and fungal populations that may interact with C. albicans to modulate the nature of the biofilm extracellular matrix. The viability of candida biofilms was significantly decreased by the presence of Pseudomonas aeruginosa and Escherichia coli. Further, it was reported
that *Streptococcus mutans* increased *C. albicans* biofilm formation, and that *C. albicans* displayed synergism with *C. glabrata* when they developed biofilm.\(^{28,29}\) The relationship between bacteria or other fungi with candidal biofilm is complex and not completely elucidated yet. It depends on the bacterial species and its numbers, and may affect the morphogenesis of the yeast.\(^{29}\)

The innate immune system has been identified as the principle protection against candidiasis. Polymorphonuclear leukocytes (PMNs) are the primary components of the innate immune response against *Candida* infections.\(^{30}\) Macrophages and neutrophils are the cells that are most commonly associated with the innate immune response against *C. albicans* infection. Macrophages produce a variety of soluble factors, including cytokines and chemokines, in response to specific microorganism, including *C. albicans*.\(^{31}\) Little is known about the ways in which macrophages and neutrophils recognize *C. albicans* as a pathogenic microorganism, or how the fungal–leukocyte interaction triggers an inflammatory response.\(^{8}\)

It was reported that viable peripheral blood mononuclear cells (PBMC) did not phagocytes the fungal cells in the biofilm form. This phenomenon is in contrast with the finding that PBMCs phagocytes the planctonic *C. albicans*. Indeed, the host innate immune response influences the biofilm formation of *C. albicans*. The biofilm formation enhancing effect of PBMCs is mediated by soluble factors, which consist of pro- and anti-inflammatory cytokines, and released into the co-culture medium of PBMCs with *C. albicans*.\(^{31}\)

**Antifungal agents resistance**

As opportunistic fungi, *C. albicans* might be found either as commensals or pathogens. The factors that facilitate its switching are considered as virulence factors. Several virulence factors were suggested, such as genes and proteins that regulate adhesion, hyphal formation, proteinase protein, phenotypic switching and biofilm formation.\(^{16}\) There is a positive association between the levels of virulence of *C. albicans* with the ability to form biofilm.\(^{24}\)

In clinical setting, biofilm forming *C. albicans* has a significant difference characteristic compared to non biofilm forming. This condition is believed as the result of distinct diseases pathogenesis. The most discussed characteristic is its resistance to antifungal treatment. It has been reported that 50% inhibition of \(^{[\text{H}]}\text{leucine incorporation (IL}_{50}\) and 50% inhibition of MTT-formazan formation (IF\(_{50}\)) for biofilms were 5 to 8 times higher than the observed values for planktonic cells and 30 to 2,000 times higher than the relevant minimum inhibitory concentrations (MIC) for five antifungals studied, i.e: amphotericin B, fluconazole, flucytosine, intraconazole, and ketoconazole. Among antifungal drugs tested in this study, fluconazole is the most effective for *C. albicans* biofilm.\(^{32}\) *C. albicans* biofilm was documented as highly resistant to antifungal agents: fluconazole, nystatin, amphotericin B, voriconazole, ravuconazole, terbenafine, and chlorhexidine in other reports.\(^{14,33}\) The resistance of *C. albicans* to antifungal agents, as reflected by its MIC was reported to increase during the development of biofilm in progress. The drug resistance develops over time, and corresponds to the development of biofilm itself, which was associated with the increase of metabolic activity of developing biofilm.\(^{14}\)

The mechanism of antifungal agents resistance in *C. albicans* biofilm is not well known yet. However, there are several reports that serve data for hypothetical mechanism of antifungal agents resistance in *C. albicans* biofilm: (1) biofilm slow growth; (2) decrease of the concentration of antifungal agents in the depth of biofilm because of penetration barrier; and (3) expression of resistant regulator genes
that associated with surface contact. One possible resistance mechanism is related to the slow growth rate of C. albicans biofilm cells as a result of the limited availability of nutrients. Slow growth is the main difference of the characteristic of biofilm forming yeast cells compared to the planktonic cells. However, the resistance to amphotericin B of C. albicans biofilm was not dependent to growth rate. It was in contrast with the susceptibility of planktonic cells to the drug that was highly dependent on growth rate. In the very low growth rate, planktonic cells were resistant to amphotericin B, but they became susceptible when the growth rate increased. This data suggested that drug resistance to antifungal drugs is not simply resulted from slow growth of C. albicans biofilm.

It was hypothesized that the biofilm’s three dimensional structures can physically prevent the entrance of antifungal agents inside the depth of biofilm, that eventually diminishes the susceptibility of biofilm against antifungal agents. Physical barrier of biofilm is mainly the consequence of deposition of extracellular matrix among the microorganisms cellular body which contributes to the biofilm formation. C. albicans biofilms which were grown in static condition and gentle shaking were compared regarding to their susceptibility to antifungal agents. Static condition is mimicking the condition with limited extracellular matrix and shaking condition is similar with condition that induces more extracellular matrix. By using this experiment, Baillie and Douglas showed that the biofilm susceptibility against antifungal agents between the two growing conditions was not significantly different. It was suggested that the extracellular matrix did not associate with the resistance of biofilm to the antifungal agents. It was also documented that the surface on which the biofilm was developed did not affect the drug susceptibility profile. However, the role of cell density in the phenotypic resistance of biofilm is in debate. There was contribution of cell density to the phenotypic resistant. However, recently it was reported that the higher number of cells does not explain the higher resistance of biofilm against biocides.

Biofilm formation results to the phenotypic change of C. albicans, which is believed to be associated with the dynamic of genes expression. Particular interest is highlighted to the genes which regulate the antifungal resistant phenotypes (TABLE 1). Understanding the genes regulation may open a new insight to the mechanisms of antifungal agents resistance associated with biofilm formation.

**TABLE 1. Genes contribute to the C. albicans biofilm antifungal agents resistance**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDRI, CDR2, MDR1</td>
<td>drug efflux activity</td>
<td>[38]</td>
</tr>
<tr>
<td>Kri1, Skn1</td>
<td>β-1,3-glucan biosynthesis</td>
<td>[39]</td>
</tr>
<tr>
<td>Erg1, 3, 6, 11, 25</td>
<td>ergosterol biosynthesis</td>
<td>[39]</td>
</tr>
<tr>
<td>Fks1p</td>
<td>β-1,3-glucan biosynthesis</td>
<td>[40]</td>
</tr>
<tr>
<td>SM11, RIM1</td>
<td>β-1,3-glucan biosynthesis</td>
<td>[41]</td>
</tr>
<tr>
<td>Hsp90</td>
<td>stabilizing regulators of cellular signaling</td>
<td>[42]</td>
</tr>
<tr>
<td>OBP, PIK, PAP</td>
<td>non sex genes</td>
<td>[43]</td>
</tr>
<tr>
<td>BGL2, PHR1, and XOGL</td>
<td>glucan transfuses</td>
<td>[44]</td>
</tr>
<tr>
<td>Gap1p</td>
<td>lipid metabolism</td>
<td>[45]</td>
</tr>
<tr>
<td>PIL1</td>
<td>the hyphal-specific echinocandin-binding protein</td>
<td>[46]</td>
</tr>
</tbody>
</table>
CONCLUSION

Candida albicans is the most frequent fungal biofilm forming yeast in the clinic. Fungal biofilm has been studied for a long time by many workers; nevertheless many questions still need to be addressed. Fungal biofilm is an inducible phenotype that may result to the antifungal agents resistance. The actual environmental and genetics make up of the yeast that plays pivotal role in this inducement is not clearly elucidated yet. Antifungal agent resistance is the most important topic, since the implication of fungal biofilm in the clinics is obvious. Molecular mechanism of antifungal resistant in biofilm is a complex pathway. We need more additional data that provide insight to find the molecular targets of drugs that may inhibit the development of biofilm in the clinical setting.

ACKNOWLEDGEMENT

Author would like to thank Ms. Mulyani for technical assistance of preparing C. albicans figure.

REFERENCES


