Biofilm: Structure, Antifungal Resistance, Proteomics and Genomics of Candida Species

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Abstract

Candida species are fungal pathogens that may cause systematic or superficial infections in the human host and threaten human health. These pathogens are also known by their ability to construct a biofilm structure on biotic or abiotic surfaces. According to their high antifungal resistance, Candida biofilms are hardly eradicated. Actually, this biofilm forming ability, protects them from antifungal drugs as same as host immune system and helps them survive under unfavorable environmental condition. Recent researches have enlightened the biofilm formation mechanism and specified many biofilm markers; in order to prevent their development. Although these studies have been helpful in so many ways, but molecular mechanisms, controlling biofilm formation and pathogenicity still remains unclear. This review focuses on information's which are already known of Candida biofilm development, antifungal resistance and genomics.

Keywords: Candida; Biofilms; Antifungal Resistance

Introduction

Biofilm, known as a biological membrane, is actually a complex, multifunctional, and multicellular structure consisting of one or more species of microorganisms, which is surrounded by a layer of inorganic and organic substances produced by the microorganisms adhered to both of the abiotic and biotic surfaces. The structure of biofilm improves the efficacy of microbial protection against the unfavorable environmental factors, such as antibiotics, decreases the efficacy of host defense mechanisms, promotes the risk of horizontal gene transfer via providing genetic and evolutionary variety, helps to facilitate the acquisition of the essential nutrients, and also enables the transmission of information between different microbial cells [1,2].

Microorganisms are able to form biofilm in different tissues in the human host or different medical devices, including intravascular catheters, joint replacements, and prosthetic heart valves, because of their ability to adhere to various types of surfaces, relating biofilms to persistent
infections and colonization [3,4]. Biofilm formation becomes an extremely important field to investigate, regarding that 80% of all microorganisms are reported to live in this form [5]. In accordance to these general features, biofilms can potentiate the establishment of some unyielding infections in their human host. This is the case of Candida biofilms, leading to systemic and superficial fungal infections in many immune compromised patients. These infections are usually very hard to treat because of the features of these species such as: expression of the virulence factors, resistance to antifungal compounds, and the potential to form biofilms. In fact, mucosal infections consist of biofilm formation, generally involving the interaction with host components and also commensal bacterial flora [6].

_Candida_ biofilms are widespread and have been noticed in most of the medical devices currently used, including shunts, stents, implants, pacemakers, endotracheal tubes, cardioverter defibrillators, cardiac devices and different types of catheters, which hinders the eradication of the _Candida_ infections. These infections might be caused by several _Candida_ spp [7,8].

_Candida albicans_ is the predominant species for Candidiasis, followed by _Candida glabrata_ [9]. _Candida tropicalis_ is usually responsible for urinary tract infections, _Candida parapsilosis_ is often observed in the skin of healthy human hosts, making it the causative agent of some catheter-related infections [10,11].

Each _Candida_ spp. reveals diversities regarding biofilm formation, mostly at the level of their features of the extracellular matrix (ECM), morphology and potential to grant antifungal resistance [2]. This diversity can increase the challenge of detecting an effective way to fight the threats of _Candida_ biofilms for human health, as a unique problem. In fact, according to the emergence of these _Candida_ infections, there is an urgent necessity to discover effective therapeutic approaches, which may have the ability to treat individuals more efficiently. The path to discover that therapeutics certainly consists of the study of the various pathogenic characteristics of these species including the biofilm formation. Although, it is a process present in all the _Candida_ spp. mentioned above, biofilm formation varies markedly from species to species, dependent to host niche, surface, and some other factors [12]. These differences can highlight the complexity of the procedures underlying biofilm formation and also the challenge to discover a unique way that will lead to the eradication of all _Candida_ biofilms. Biofilms generally occur in the endothelium or mucosa evidenced to be involved in the development of common candidiasis, including oral and vaginal candidiasis, but also correlated with medical devices, including dentures and urinary and vascular catheters [2,6]. Since _Candida_ biofilm-related infections exhibit many economic and clinical consequences, recent studies about the pathogenicity of _Candida_ spp. have mainly focused on the management and prevention of biofilms. The current review defines the chief aspects of what is presently known about _Candida_ biofilm regulation and development.

**Biofilm Formation**

Our conception of _Candida_ biofilm development and structure is based on observations made employing different microscopic techniques such as fluorescence microscopy, confocal scanning laser microscopy, and scanning electron microscopy [2].

The procedure of biofilm formation is multistage and depends on the properties of the microorganisms, construction, and properties of the colonized materials or the host. There are four basic phases (A-D), as illustrated in Figure 1.

![Figure 1: Biofilm formation phases.](image)

The beginning of formation of biofilm is with the adhesion of free floating microorganisms to the bionic or abiotic surface. Reversible adhesion appears as the result of weak physical interactions causing the first cells to be
attached to a solid surface such as electromagnetic surface charge, gravitational interaction, electrostatic, van der Waals forces, and hydro/thermodynamic forces (Brownian motion). These kinds of forces exert a vital role when there is large surface and distance between cells. Biofilms are impermanent and can be easily removed by both physical and chemical techniques. There is irreversible adhesion due to the formation of specific bonds, when there is less than 1.5 nm between cells and the surface. First microbial cells that are attached to the surface help other ones to attach by the formation of hydrophobic, non-specific or specific hydrogen bonds, additional to pairs and ionic complexes (carbon-carbon covalent bonds) [13-16].

An important place in the process of biofilm formation is the interaction of adhesives, specific receptors, and ligands on the cell surface of the microorganism or the extracellular ligand of the host cell. At first, a single layer of microbial cells covers the surfaces. In the process of making the basic EPS matrix, which gives the biofilm a specified structure and shape, an increase in the secretion and synthesis of extracellular biopolymers exerts an important role. Biofilms are produced by increasing the severity of cell proliferation. While, glycocalyx, which is a shell made of polysaccharide residues of glycoproteins and glycolipids of cell membrane, is expanded up to the whole surroundings of the micro colonies. At this stage, biofilm also includes dead cells, organic compounds, and mineral substances, in addition to living microorganisms. Microbial cells can join these parts. Irreversible adhesion allows the micro colonies formation and maturation of biofilm [14-16].

The microorganism reproduction, their gradual differentiation and the inhibition or activation of expression of certain genes can induce biofilm maturation. Biofilm cells obtain characteristics which are not demonstrated by planktonic cells and can transmit them to progeny and adjacent cells. When the membrane of biofilm reaches the critical thickness, we can have cell migration from peripheral parts of the mature biofilm to the surrounding environment and then the process of colonization begins. Due to a reaction to adverse environmental conditions, cells from biofilm disconnect. This dispersion is an intentional technique. Biofilm adapts to environmental pressures and the cells that have been detached start the process of colonization of new surfaces [1,14-18].

*Candida* biofilms forming in *in-vivo* systems seem to pursue the same sequence [19]. In spite of the fact that, thickness is greater and maturation is faster in these biofilms than in those grown in *in-vitro* models. The range of thickness of a biofilm formed *in-vitro* can alter from 25 mµ to 450 mµ whereas it usually exceeds 100 mµ in *in-vivo* systems [2,19-21].

Biofilms are different in their matrix and structure composition, differing among strains and species. Regarding *C. albicans*, the biofilm structure is typically made up of two layers; a basal deposit of blastopores, which is covered by a thick matrix film with hyphal forms. Moreover, biofilm production in this species, as previously mentioned, is correlated with the transition from yeast to hyphal growth. Comparing to *C. albicans*, *C. parapsilosis* biofilms are much less thick, composing of aggregated blastopores additional to yeast cells and pseudo hyphae. Regarding *C. tropicalis*, the mature biofilms are generally identified through their dense network of yeast cells with evident filamentous morphologies. Contrary to this species, biofilms of *C. glabrata* are distinguished via compact multilayer or monolayer with only blastopores, for the reason that this species is not capable of forming filamentous forms (Figure 2) [22].

Figure 2: Scanning electron microscopy (SEM) images of 24 h *Candida* biofilm growth. 
A: *Candida albicans*; B: *Candida parapsilosis*; C: *Candida tropicalis*; D: *Candida glabrata* 
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Factors Affecting Biofilm Architecture and Formation

Formation of biofilm is affected by various host and different Candida-derived variables, such as nutrients, fluid flow, microbial products, and host receptor.

Substratum

Numerous in vitro model systems, including silicone elastomer catheter disks, acrylic, cellulose cylindrical filters glass, polymethylmethacrylate, and plastic have been employed to develop Candida biofilms [12,23,24]. Biotic surfaces, including those in an engineered human oral mucosa model, have been reported to be employed as a substratum [25]. This reveals that various substrates can greatly affect the morphology, thickness, and architecture of biofilms. In a previous study, Douglas and Hawser evaluated different catheter materials and revealed that C. albicans biofilm formation was slightly increased on silicone or latex elastomer (P<0.05) comparing with polyvinylchloride but was substantially reduced on 100% silicone or polyurethane (P<0.001) [26]. Scanning electron microscopy exhibited that after 48 h, C. albicans biofilms involved a dense network of germ tubes, yeasts, hyphae, and pseudo hyphae; moreover, extracellular polymeric material was observed on the surfaces of some of those morphological forms.

In a recent investigation, Estivill, et al. [27] studied biofilm formation by 84 strains of 5 Candida spp. on 3 clinical materials and the results revealed that all of the tested Candida strains had the ability to form biofilms. Furthermore, all species demonstrated higher capacity to form biofilm on Teflon, except C. glabrata that exhibited greater capacity to form biofilm capacity on polyvinyl chloride. All together, these researches exhibited that the capacity of Candida to form biofilms is highly affected by the type of material on which it is growing and also on the species of Candida.

Nutrients

Nutrients in the growth media, such as lipids, sugars, and serum, are vital determinants of the Candida biofilm-forming capacity. In a previous study, the effects of sucrose on the colonization of acrylic by C. albicans in both mixed and pure culture in an artificial mouth was evaluated [28]. The results revealed that the number of Candida cells was markedly increased on acrylic which was exposed to sucrose, while the number of the salivary bacteria was not altered by sucrose. In another research, the growth of C. albicans biofilms in medium containing 50 mm glucose or 500 mm galactose reached the peak after 48 h and hence decreased; nonetheless, the cell yield was reported to be lower in the medium with low glucose [26].

Other investigations have demonstrated that fructose, lactose, and glucose favor the formation of C. albicans biofilm comparing with the other dietary sugars including maltose and sucrose [29]. In another study, Swindle, et al. [30] investigated the effect of parenteral lipid emulsion on Candida biofilms which were formed on the medical catheter surfaces. Biofilms, which were formed on silicone-elastomer catheter discs, were analyzed via confocal laser microscopy and scanning electron microscopy. Adding lipid emulsion to a standard growth medium leaded to an increase in the production of C. albicans biofilm and altered biofilm architecture and morphology. Moreover, lipid emulsion assisted in the growth of C. albicans and gave rise to germination. These results account for the increased risk of candidemia in individuals who receive lipid emulsion through medical catheters. A recent study conducted by Samaranayake, et al. [31] Confirmed that human serum can promote the growth of C. albicans biofilm on silicone biomaterial and it may also induce the expression of genes correlated with production of hydrolase (PLB2, PLB1, and SAP) and adhesion (HWP1 and ALS3).

Fluid Flow

Physiological conditions such as fluid flow at the site of infection are major modulators of biofilm, because the liquid flow may affect structural integrity and the exchange of nutrient in biofilms [32]. Many researchers have tried to mimic these conditions in vitro, such as mimicking the flow of blood, saliva, and urine, additional to the application of continuous flow cells to investigate the fungal biofilms. In that regard a previous study evaluated the ability of C. tropicalis and C. albicans to form biofilms on silicone rubber voice prostheses in the presence or absence of a salivary conditioning film situated in a parallel-plate flow chamber, and exhibited that biofilms, which were formed under flow in the presence of a salivary film were more likely to detach faster than those, which were formed directly on the substratum [33]. In contrast to this finding Jin, et al. [24] has demonstrated that regardless of dietary sugar supplementation, the presence of a salivary coating does not markedly affect biofilm formation. These contrasting results can be because of method of sample collection, use of saliva, and diversities in the nature (for example the quality of saliva derived from different patients).

Other researchers employed the parallel-plate flow chamber to investigate the construction of Candida-
bacteria mixed biofilms on acrylic and glass. Zimmermann, et al. [34] in another study, employed the continuous flow culture to demonstrate that when testing under anaerobic conditions, voriconazole and fluconazole show cidal activity; however these agents were all static against Candida biofilms, under aerobic conditions. Tyler and Suci reported an in situ method to assess the function of chlorhexidine against Candida biofilms in a flow cell system via monitoring the penetration of the kinetics of propidium iodide (PI) into the cytoplasm of the individual cells while dosing with chlorhexidine [35]. This model permitted monitoring of the rate of propidium iodide penetration into different subpopulations (hyphae vs. yeast) of the biofilm. Researchers have also employed some airflow models to investigate voice prosthesis, because the obstruction of airflow is an important cause of prematurity and early replacement of the mentioned devices [7].

**Microbial Cohabitants**

The ability of Candida to construct biofilm is influenced by the presence of other Candida spp. additional to the presence of different bacterial cohabitants. Reid, et al. [36], in a recent study revealed that the ability of Candida spp. to construct biofilms on epithelial cells and fibers is influenced by Lactobacillus. While lactobacilli exposure exhibited up to 91% displacement of preformed C. albicans biofilms, fibers, which were precoated with lactobacilli, actually inhibited the Candida adhesion by 0 to 67%. Experiments with epithelial cells demonstrated that the lactobacilli might markedly interfere with the adhesion of Candida to the cells. This suggests that members of normal female urogenital flora could interfere with infections, which are caused by Candida.

Holmes, et al. [37] proved that C. tropicalis and C. albicans, two frequent oral fungi, bind to Streptococcus gordonii, but the other two Candida species (C. kefyr and C. krusei) do not. Furthermore, there was a positive association between the ability of Candida to adhere to experimental salivary pellicle and adherence to S. gordonii. Depending on streptococcal growth conditions, whole saliva either slightly inhibited or stimulated adherence of C. albicans to S. gordonii. El-Azizi, et al. [38] investigated the interactions between C. albicans and twelve other species of Candida additional to bacteria in the biofilms and proved decreased biofilm production by C. albicans at the time the fungus was added to preformed biofilms of bacteria and non-albicans Candida. Although, when Staphylococcus epidermidis (a nonglycocalyx producer), C. parapsilosis, or Serratia marcescens was added to the preformed C. albicans biofilms, the number of the cells of the added microbes rose in the growing biofilms, exhibiting a dynamic interaction between biofilms of C. albicans and other fungi and bacteria.

In a recent study, Park, et al. [39] demonstrated that co-culturing with bacteria reduced the ability to form biofilms in C. albicans. In another study, van der Mei, et al. [40] investigated the ability of C. tropicalis and C. albicans to produce biofilms on silicone voice prostheses in the presence and absence of Lactobacillus strains and various commensal bacterial strains. They exhibited that biofilms, which consisted of combinations of a bacterial strain and C. albicans comprised markedly less viable organisms than those combinations that comprised C. tropicalis. Furthermore, high percentages of Candida were observed in biofilms that were grown in combination with lactobacilli. The procedures underlying the mentioned interactions within biofilms of Candida have been proposed to consist of microbial proteins (for example proteins produced by bacteria additional to Candida proteins) as well as the host products (for example salivary adhesins) Researches have also demonstrated that various P. aeruginosa virulence factors, such as phenazine (e.g., pyocyanin), and homoserine lactones are related to the inhibition of biofilms of Candida [41-43]. These Researches reveal that fungal-bacterial and fungal-fungal interactions exert key roles in modulating the ability of Candida to produce biofilms. How the mentioned interactions are related to varieties in microbial communities (mycobiome and bacteriome) in a biofilm is a topic that has not been well studied and demands a lot more investigations.

**Variability of the Species**

The ability to produce biofilms can differ widely among strains of Candida. In accordance to this, in a previous study, Branchini, et al. [44] produced pulsed-field gel electrophoresis and electrophoretic karyotyping to reveal slime production and genotypic variation among 31 isolates of C. parapsilosis derived from individuals with catheter infections or bloodstream. A total of fourteen DNA subtypes were detected among the 31 isolates, known that almost 80% of which constructed biofilms; the ability to form biofilm among the strains altered from moderate to strong (67%) and weak (13%). Kuhn, et al. [45], in a recent study compared the biofilms produced by C. parapsilosis and C. albicans on catheter surfaces employing dry weight assays and XTT, followed by confocal scanning laser microscopy and fluorescence microscopy. These researchers confirmed marked differences in biofilm formation between noninvasive and invasive isolates of C. albicans; C. albicans isolates formed more biofilm than C. tropicalis, C. parapsilosis, and C.
Compared biofilm production by 15- is nit, preterm neonates, transplant recipients served for all three tested (C. tropicalis) result in an accumulation of the ow an increase in fluconazole ow of, which is C. albicans, C. dubliniensis, C. glabrata, C. port scored for all three isolates of C. albicans and the ability to form biofilm revealed a trend of lower thickness of biofilm species. Scanning confocal and electron microscopy produced biofilms to the same extent. Although, measured by XTT and biomass assays, all three species showed that was high in both carbohydrate and protein content. Interestingly, matrix of biofilm way around, matrix, that were obtained from large amounts of carbohydrate and less protein. The other showed that greatly strain dependent. Scanning electron microscopy parapsilosis, C. parapsilosis, C. orthopsilosis, C. parapsilosis, and C. glabrata) were reported to recover from different sources, employing crystal violet staining [46].

All non-albicans Candida species had the ability to form biofilms, in spite of the fact that these were less extensive for C. glabrata than C. tropicalis and C. parapsilosis, and C. parapsilosis biofilm formation was greatly strain dependent. Scanning electron microscopy showed that C. parapsilosis biofilm matrix consisted of large amounts of carbohydrate and less protein. The other way around, matrix, that were obtained from C. tropicalis biofilms consisted of low amounts of protein and carbohydrate. Interestingly, matrix of C. glabrata biofilm was high in both carbohydrate and protein content.

Biofilm production by ten clinical isolates each of C. orthopsilosis, C. parapsilosis and C. metapsilosis were characterized by Lattif, et al. [36]. They reported that as measured by XTT and biomass assays, all three species produced biofilms to the same extent. Although, variations dependent to strain in the metabolic function of produced biofilms was observed for all three tested species. Scanning confocal and electron microscopy exhibited that while the three species produced biofilms with similar architecture and topography, C. metapsilosis biofilms revealed a trend of lower thickness of biofilm comparing to C. orthopsilosis and C. parapsilosis. All together, these results exhibited that the ability to form biofilm, matrix composition, and structure are greatly depended to species. Generally, C. albicans forms more complex and larger biofilms than other the species.

**Antifungal Resistance**

Increased antifungal resistance of Candida spp., demonstrated in the biofilm mode of growth, was first discovered by Hawser and Doula [2].

One of the most dangerous clinical aspects of main Candida spp. (C. albicans, C. dubliniensis, C. glabrata, C. krusei, C. tropicalis, and C. parapsilosis) is their capacity to form biofilm. The clinical importance of biofilm is growing with the increasing production of different medical devices that have been worked on the human body. Approximately all the contaminations of the devices mentioned before, is the result of their colonization by microorganisms. This can facilitate organization of biofilm structures [1].

The main classes of antifungal compounds, which are used to tackle Candida spp. infections, are azoles, polyenes, and echinocandins [47-50]. Azoles (e.g. voriconazole, fluconazole, and posaconazole) with a fungistatic effect, blocking ergosterol synthesis, targeting the enzyme lanosterol 14α-demethylase (related to the ERG11 gene) result in an accumulation of the intermediates of toxic sterol pathway. Polyenes (e.g; amphotericin B and nystatin) are fungicidal, attaching to membranes including ergosterol, creating pores that can destroy the proton gradient, which lead to the outflow of the cytoplasm and other cell substances. Echinocandins (e.g; micafungin, caspofungin, and anidulafungin) are also kind of a fungicidal, which can interfere with the synthesis of 1,3-β-glucan, a component of the cell wall of Candida spp. It is also important about azoles that the application of polyenes and echinocandins is suggested if the patient had prior azoles exposure and if the infection is severe for patients have been infected with C. glabrata, which is known as frequent azole-resistant. Echinocandins are most often and the first antifungal drug choice in these vigorous cases of candidemia, in accordance to the recent guidelines [51,52]. Several studies prove that prophylactic application of fluconazole may have positive effects for severe care unit, preterm neonates, transplant recipients patients, and other high-risk patient populations [53-55]. Though, because of some controversies, this is not considered as a general standard for all hospitals [21].

Since fluconazole is used as the main antifungal drug for HIV/AIDS patients, fluconazole resistance has been explored in several previous articles. In vitro fluconazole resistance of Candida biofilms is in the range of 250 to 400 times that of planktonic Candida [20]. In vivo, Candida biofilms also show an increase in fluconazole resistance; biofilms of Candida had a minimum inhibitory concentration (MIC) for fluconazole, which was 128 times as high as that of the planktonic Candida [20,56]. This feature has clinical implications, but a huge hope has been raised with the development of newer antifungal components, such as echinocandins and liposomal forms of amphotericin B. Some studies have explained that the
latter antifungals can be effective against Candida biofilms [2,57].

Studies have demonstrated that Candida species. Biofilm has some resistance to fluconazole at a concentration of 2000 times higher than the value of MIC for the planktonic form. Echinocandin and the liposomal formulation of amphotericin B are the most active antibiotics against Candida sp, biofilm. These agents demonstrate anti-biofilm function in concentrations 2-25 times higher than the MIC values against the planktonic forms [56,58] There is also the possibility of producing different mechanisms of drug resistance by each cell in the biofilm [1].

Factors Affecting Antifungal Resistance

Multiple factors have been suggested for increasing antifungal resistance of Candida biofilms. These involve metabolic /growth ratio of the biofilm cells, presence of the persisted cells, expression of the resistance genes, and also presence of the extracellular matrix [2].

Biofilm Growth

Although there is connection between growth rate and resistance of bacterial biofilms, Baillie and Douglas showed that growth rate is not related to Candida biofilm resistance to amphotericin B. But, the susceptibility of planktonic cultures is the result of their growth rate [59]. As a result, antifungal resistance of Candida biofilms is not significantly correlated with the reduction of the cell growth rate [20].

A previous study did compared the MIC values of nystatin, amphotericin, B fluconazole, and chlorhexidine antifungals during early, intermediate, and maturation phases of Candida biofilms. Development of drug resistance of the Candida biofilm was correlated with huge increase in metabolic function of the expanding biofilm. This conception also proved that increased drug resistance is not basically a result of lower metabolic function of cells in maturing biofilms, but is more associated with the maturation procedures [12].

Extracellular Matrix

Generally, the extracellular polymeric substances (EPS) can act as a physical barrier that prevents the access of antimicrobials to cells imbedded in the biofilm community, in turn leading to advanced drug resistance. This barrier depends mostly on the nature and amount of the EPS, and also the physicochemical characteristics of the drug. In bacterial biofilms, EPS enzymes digest drugs, too. The role of the EPS in the resistance of Candida biofilm is obscure. In one of the studies in this field, the survival rate of Candida cells in biofilms that had been treated with amphotericin B decreased about 20% when the EPS was removed [60].

In another study, C. albicans biofilms that are formed under a permanent liquid flow were being stimulated to increase synthesis of matrix to an extent that improved the resistance against amphotericin B markedly [61]. This field of research is surely a valuable source for future studies and has several implications as EPS in clinical fields or its components may be employed as possible drug goals [2].

Genetic Basis of Antifungal Resistance

Molecular mechanisms, which confer superior antifungal resistance in Candida biofilms, are not completely elucidated. Studies have displayed the involvement of ATP-binding cassette and main facilitator superfamily pumps of drug efflux in increased resistance to azole antifungals. However, efflux pumps lead to azole resistance only in the early stages, but not in the later phases, of Candida biofilm growth. Moreover, membrane sterol compounds may lead to azole resistance in the mature and intermediate phases. In this connection, CDR1, CDR2, and MDR1 genes are being up-regulated in the biofilm growth [2].

Persisted Cells

Rather than mutants, persister cells are among phenotypic variants of wild-type cells and continue to live despite the presence of antibiotics at some concentrations more than the MIC [59]. Bacterial biofilms produce recumbent persister cells known to be responsible for the tolerance against drugs, and have been observed in biofilms of Staphylococcus aureus, Escherichia coli and, Pseudomonas aeruginosa. A subpopulation of highly antifungal tolerant cells was found in Candida biofilms; however, these cells could not be found in cultures of Candida planktonic [62].

In the recent studies, Candida biofilms demonstrated a biphasic killing pattern responding to microbial factors. The surviving subpopulation of persister cells have demonstrated multidrug tolerance, while others were susceptible to the antifungals studied. Reaggregation of surviving cells can construct a new biofilm with a new persister cells subpopulation, hence proving that persister cells are phenotypic variants of the wild type with a genotype, which has the ability of heritage. This area of study about biofilm biology requires a significant
focus and a lot more studies has to be conducted to explore this phenomenon [2].

**Proteomics and Genomics**

Proteomics and genomics are broad disciplines that have appeared as necessary sciences for understanding molecular basis of any pathophysiological phenomenon. However, the exact genetic discussions are beyond the purpose of this article [2]. In this regard, we decided to outline the most relevant genomic and proteomic findings related to *Candida* biofilm. Genomic researches on *Candida* biofilm have gathered invaluable information about mechanisms, which are controlling biofilm formation. Several methods have attempted to determine the genetic mechanism behind *Candida* biofilm formation. Researchers have investigated the result of single deletion mutation, by performing systemic searches using mutant collections running transcriptomic analyses or running individualized transcription factor analyses [63-67].

The specific hypotheses related to biofilm formation have prompted the study of some genes [68]. For example, hyphae are important factors in *C. albicans* biofilms, hence it was assumed that hyphae are essential to form a biofilm and the transcription factor genes *CPH1* and *EFG1* are positive factors in hyphae morphogenesis regulation; while *efg1/efg1 cph1/cph1* mutant is incapable of forming a normal biofilm [67,69]. Likewise expression profiling confirmed that many amino acid biosynthetic genes were regulated, in higher orders, during biofilm development, and also the fact that amino acid activator GCN4 mutation decreases biofilm biomass claiming that expression increase of those genes is practically considerable for biofilm formation [64].

Other genes were also identified through genetic screens. As an example, a random insertional mutagenesis confirmed that KEM1, MDS3, NUP85 and SUV3 are necessary for in vitro biofilm formation [63]. Although there was no previous functional connection between these genes, they all turned to be necessary for hyphal development, which confirms the hypothesis that hyphal formation is necessary for biofilm formation. For second example, it was revealed that nonsex genes in mating type locus of *C. albicans* are related to a/α biofilm formation. The mating type locus (MTL) of *C. albicans* includes the mating type genes playing a particular role in the mating process; but this locus also includes 3 seemingly unrelated “nonsex” genes (NSGs) such as PIK, PAP and OBP, the first 2 are necessary for growth. By mutational analysis, it is illustrated that bothering the nonsex and mating type genes in MTL are important in a/α biofilm formation, and that OBP is necessary for fluconazole resistance and impermeability [70].

There were also some studies focusing on biofilms in particular kinds of candidiasis. For example, ocular *C. albicans* isolated from patients with microbial cellulitis and keratitis showed different susceptibility to antifungal agents, and some of them had ability to form a biofilm. Multiple genes related to biofilm formation, pathogenicity and drug resistance were regulated in biofilm-forming *C. albicans*, in comparison with non-biofilm-forming *C. albicans*. Temporal gene expression in biofilm-forming species was helpful in order to find potential necessary genes in different stages of biofilm formation; these genes may serve as probable targets for biofilm formation and resistance to antifungal disturbance [71].

Proteomics is a branch of biology science that studies “protein complements of genome” [72]. *Candida* proteomic studies have mostly been restricted to cell wall analysis and assessment of proteomic changes related to drug responses, change in pathogenicity of mutants, and serological response to candida infection [73]. While, there have been less studies on *Candida* biofilms in comparison. Interestingly, according to former studies, there is a remarkable similarity between biofilm and planktonic proteomic [74].

In one study, it was noticed that non-glucan attached proteins of the extracellular matrix of *C. albicans* biofilms and the cell surface are usually similar to planktonic yeast proteins [75]. There also have been some studies focusing on some specific proteomic aspects. For example, proteomic analysis of ergostrol, sphingolipid and oxidative stress pathway modulation by myristic acid (MA) have been conducted. According to this article, proteins related to glucosyl ergostrol and oxidative stress pathway is necessary for *C. albicans* to conflict with host immune system in order to survive. The negative regulation of those proteins proofed the antifungal activity of MA against *C. albicans*. As a result, MA can be considered as an ideal candidate for combination therapy with fluconazole [76]. In the end, we can briefly say that getting familiar with genomic and proteomic basis of biofilm formation in candida species can be extremely helpful with developing further treatment strategies.

**Conclusion**

The main pathogenic characteristic that helps *Candida* spp. to cause disease in human host is biofilm formation. Biofilm development, architecture and antifungal resistance mechanisms have been the major purpose of
former researchers. In general, biofilm cells in comparison with planktonic cells are less sensitive to antifungal agents and have a higher survival rate. Increasing development of microscopic, genomic and proteomic tools are really helpful with picturing a vivid overview of molecular basis governing pathogenic mechanism of Candida biofilm formation. Such information can give us invaluable clues, in order to help us combat this ubiquitous fungus.

References


