Clinical and pathogenicity aspects of *Candida* species

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Candida species normally exist as commensals of the skin and mucosa, but they are also opportunistic pathogens, with the ability to cause a variety of superficial and invasive diseases. Among the several of *Candida* species, *Candida albicans* remains the most common fungal isolate, mainly from blood cultures in the diagnostic of nosocomial bloodstream infection. However, the rates of candida infections caused by other species have increasing significantly in the last years. The pathogenesis of the candidiasis is influenced by the predisposing factors of the host and virulence factors expressed by the yeast involved in the infection. In this way, various characteristics of *Candida* species have been proposed as virulence factors that enable fungal adhesion, colonization and subsequent penetration of tissues in susceptible hosts. In this regard, secretion of hydrolytic enzymes such as lipases, aspartyl proteinases and hemolytic factor contributes to candida pathogenesis by degrading cell membranes and extracellular proteins. In addition, the ability to recognize and adhere to host cells and/or tissues and biofilm formation has been implicated as potential virulence factors for at least one *Candida* species

Keywords *Candida*; virulence factors; biofilm production; hemolytic activity; phospholipase; proteinases; adherence; switching phenomenon

1. *Candida* spp - Clinical Importance and Epidemiology

Species of the genus *Candida* are distributed in the environment and microbiota of the skin and mucous membranes of humans and animals [1-4]. *C. albicans* is the most prevalent pathogenic species; however, non-*albicans* species such as *C. parapsilosis sensu stricto*, *C. orthopsilosis*, *C. metapsilosis*, *C. tropicalis*, *C. glabrata, C. krusei*, *C. dubliniensis, C. famata, C. guilliermondii, C. lusitaniae, C. rugosa,* and *C. zeylanoides* have gradually increased as major agents of infection in humans [5-11].

Candidiasis is an opportunistic fungal infection and installs due to imbalance between microorganism and host interaction [12], causing clinical manifestations that can range from superficial lesions, such as cutaneous and mucous membranes, up to a serious and systemic affliction characterizing invasive candidiasis [3,9,13-16]. Superficial candidiasis is classified into four clinical types according to the location and extent of involvement: nail, cutaneous, mucosal (buccal, vulvovaginal, and balanopreputial), chronic mucocutaneous, and granulomatous [17].

Candida species are the main etiological agents of onychomycosis, a superficial infection that affects fingernails and toenails, causing nail dystrophy with inflammatory reaction in the nail folds [18-23]. Some studies demonstrate a varied distribution of *Candida* species related to onychomycosis. In a study carried out in Iran, Hashemi *et al.* 2010 [24] reported candidal nails in 216 individuals, and *C. albicans* was the most isolated agent. Vasconcelos *et al.* 2013 [21] showed that *Candida* species were the second most common cause of onychomycosis in elderly institutionalized individuals in *São Bernardo do Campo*, *São Paulo*, Brazil, and *C. guilliermondii* followed by *C. parapsilosis* were the most isolated species. In the Central West region of Brazil, in the state of *Goiás*, Ataides *et al.* 2012 [19] diagnosed 53 cases of nail candidiasis, and the main etiological agent was *C. parapsilosis.*

Cutaneous candidiasis mainly affects intertriginous areas. Maceration of epithelial tissue, heat, and high humidity are important conditions in the development of lesions with erythematous, moist, or even squamous and/or pustular characteristics [17, 25]. Emerging non-*albicans* species have been described as agents of surface infections by Flores *et al.* 2009 [26] in Peru, where they showed the frequency of *C. krusei, C. tropicalis* and *C. glabrata.* At a dermatology reference center in Singapore, Tan [27] reported that cutaneous candidiasis accounted for 11.1% of 12,923 cases of superficial infections, and *C. albicans* (65%), *C. glabrata* (23%), *C. tropicalis* (7%) and *C. parapsilosis* (5%) were the main species identified.

The isolation of *Candida* species from buccal mucosa does not mean the presence of an infection, because these yeasts are the main microorganisms in the microbiota of the mouth [20, 28]. The transition for *Candida* to a pathogen is due to predisposing factors of the host, such as immunodepression, because of HIV infection, premature newborns, breastfeeding mothers, and the elderly [29-31]. The clinical manifestations that characterize oral candidiasis are variable and classified as pseudomembranous, angular cheilitis, erythematous, and leukoplakia or chronic hyperplastic candidiasis [28, 32].

C. albicans is the most frequent species in oral candidiasis, although other species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis, C. krusei*, *C. kefyr* and *C. dubliniensis* are emerging as agents of this infection [33, 30, 31]. In India, Mane & Pratyusha 2013 [31] reported that individuals undergoing chemotherapy or radiotherapy are more predisposed to develop oropharyngeal candidiasis due to induction of oral mucositis by the treatment. These authors verified that *C. albicans* was the etiological agent in 54.2% of the cases, but other non-*albicans* species such as *C. tropicalis, C. krusei, C. glabrata* and *C. parapsilosi*s were isolated from these patients.

Infections involving the genitourinary system - vaginal mucosa and balanopreputial - are very frequent clinical conditions. The characteristic manifestations include vulvovaginitis, balanoposthitis, and candiduria, which can be observed in both genders [34]. Clinical symptoms that characterize vulvovaginal candidiasis (VVC) are non-specific, presenting an inflammatory process with thick white secretion, vulvar pruritus, dysuria and dyspareunia. Balanoposthitis is an inflammation of the glans and foreskin of the penis, with a burning sensation, pruritus, pain and sometimes, whitish lesions with secretions [17, 34]. The presence of *Candida* yeasts in urine (candiduria) may represent a urinary tract infection with episodes of fever, urinary urgency, polaciuria, and dysuria [1, 34].

Among the main gynecological problems, VVC is the second most common cause of infection, exceeded only by bacterial vaginosis [35, 36]. *C. albicans* is the main etiological agent, whereas among non-*albicans* species *C. glabrata* and *C. tropicalis* are frequently responsible for this clinical condition [37, 38]. *Candida* species account for approximately 30-35% of the causes of infectious balanoposthitis [34, 39]. The diagnosis is based on clinical information; however, it is believed that the establishment of this infectious process is related to sexual contact, since the main agents of candidiasis in the male genital tract are similar to those observed in cases of VVC [34].

Among the infectious agents that affect the urinary tract *Candida spp* are found in about 10-15% of the cases [40-42].

The use of a bladder catheter associated with prolonged periods of hospitalization, antibiotic therapy, diabetes mellitus, and advanced age are among the most important predisposing factors for infection [43, 44]. *C. albicans, C. glabrata, C. tropicalis* and *C. parapsilosis* are the most frequently isolated species. Zarei-Mahmoudabadi *et al* 2012 [44] in Iran, observed *C. albicans* (53.3%) as the main etiological agent of candiduria in hospitalized patients, and among non-*albicans*, *C. glabrata* (24.4%), *C. tropicalis*, (3.7%) and *C. krusei* (2.2%) were the most frequent; antibiotic therapy was the main predisposing factor. In individuals with kidney transplants, *C. glabrata* (49.4%) was the main agent of candiduria, followed by *C. albicans* (19.2%), *C. krusei* (8.4%) and *C. tropicalis* (8.4 %). According to Fraisse *et al* 2011 [40] , urinary infections in patients over 85 years old at a university hospital in France had an 8.9% prevalence of candidiasis, with *C. albicans* isolated in 59% of cases, followed by *C. glabrata* (24%), *C. kefyr* (3%), *C. lusitaniae* (2%), *C. krusei* and *C. tropicalis* (1%). However, in Turkey, Ozhak-Baysan *et al* 2012 [45] reported that non-*albicans* species were the main agents of candiduria (56%), with *C. tropicalis* and *C. glabrata* accounting for 38%.

Chronic and granulomatous mucocutaneous candidiasis caused mainly by *C. albicans* represents a complex group of recurrent or persistent manifestations, which can affect the face, skin, scalp, and hands simultaneously [46]. This clinical type generally progresses to a severe condition, with lesions characterized by hyperkeratosis with thickening of the skin and subcutaneous tissues [17, 47]. Immune system factors, such as functional or quantitative alteration of T lymphocytes and γ-globulin deficiency, are important in the progressive evolution of the disease [48, 49].

The increase in invasive fungal infections is mainly due to cases of candidiasis [50-54]. Candidemia has been considered one of the leading fungal infections among Intensive Care Unit (ICU) patients worldwide, with a relatively high frequency, ranging from 7 to 17.8% [55-60]. An important factor that contributes to this high frequency is the immunocompromised state of ICU patients that favors penetration and installation of the microorganism through exogenous transmission through contact with health professionals or contaminated hospital medical materials [60-63]. In addition, a worse prognosis is related to broad-spectrum antibiotic therapy, prolonged use of central venous catheter, parenteral nutrition, and digestive tract surgery [54, 62, 64, 65].

It is difficult to explain the progressive increase of non-*albicans* species linked to invasive manifestations of candidiasis. However, it is known that *C. parapsilosis* and *C. glabrata* are related to some factors that allow the development of invasive infections, such as central venous catheter and prophylactic use of antifungals [13, 53, 61, 66-70]. The use of a central venous catheter is an important factor in the development of systemic infections, because it allows the invasion in the host mainly by species that have a greater capacity to form biofilm, such as *C. parapsilosis sensu stricto* [15, 60, 62, 71, 72]. Prophylactic measures with azole derivatives, mainly fluconazole, are used for *C. albicans* infections; however, other species resistant to this drug, such as *C. glabrata*, have emerged as infectious agents [73-75].

2. Virulence factors

The main virulence factors related to the pathogenicity of *Candida* species include the ability to produce and secrete hydrolytic enzymes, such as proteases, phospholipases, and lipases; expression of adhesion factors and biofilm formation; switching phenomena and hemolytic factors [3, 10, 15, 23, 76-81]

The recognition of these virulence factors elucidates characteristics of the pathophysiology of candidiasis, as different yeast responses are observed, according to the anatomical site of infection and conditions of the host. Synergistic action among these factors can occur, permitting more aggressive colonization of cells and tissues [10, 3, 82, 83]. *C. albicans* is the main species used to demonstrate virulence factors expressed *in vitro* and *in vivo*; however, other species are also capable of expressing these factors, raising the importance of these characteristics that determine the virulence of the microorganism.

2.1 Proteinases

Proteinases degrade cell surface structures and act by inhibiting the functions of the host's immune system [81-83]. In this way, a relation between the increase of the synthesis and activity of the proteinases increases the pathogenic potential of the yeasts, contributing to a more severe evolution of the candidiasis in clinical situations [3, 10, 84, 85].

The secreted aspartyl proteases (Sap) secreted by *C. albicans* are exoenzymes with molecular mass between 35 and 50 kDa, which are expressed by ten genes, *SAP1-10* (SAP - Secreted Aspartyl Proteinase), included in six subfamilies *SAP1-3*, *SAP4-6*, *SAP7*, *SAP8*, *SAP9*, and *SAP10* [10, 86]. SAPs 1-6 genes are related to adhesion, damage, and tissue invasion. An important condition for the action of exoenzymes is pH. Saps 1-3 have a more effective action at pH ranging from 3.2 to 4.5, while Saps 4-6 perform best at pH 5.0. The functions of Sap 7 and Sap 8 are not fully understood, but it is known that the switching phenomenon of *Candida* species occurs at a temperature of 25 °C, probably due to expression of the *SAP8* gene. The production profile of the exoenzymes, Saps 9-10 has not yet been fully evaluated, but experiments demonstrate that the *SAP*9-10 genes correlate with the preservation of cell wall integrity of the yeast [10, 81, 83, 87].

C. parapsilosis and *C. tropicalis* usually have high extracellular proteolytic activity *in vitro* [10,15]. Three genes (*SAPP1-3*) encoding extracellular Saps have been identified for *C. parapsilosis*, with Sapp2p constituting about 20% of the Saps found in culture supernatant of the related species [15, 88, 89]. The involvement of Sapp in the pathogenesis of *C. parapsilosis* infections has not been yet fully elucidated, but there is usually a correlation between the isolation site, the species of the complex, and the amount of enzyme produced. Greater secretion is observed in isolates of superficial infections, compared to those of invasive infections [15, 81]. Tosun *et al* 2013 [89] found that *C. parapsilosis sensu stricto* was the only Sap producing species, and a higher proteolytic activity was verified in urine isolates than blood isolates. However, Ge *et al* 2011 [90] and Treviño-Rangel *et al* 2013 [91] verified a strong *in vitro* activity of Saps in *C. metapsilosis* isolates.

C. tropicalis has four genes (*SAPT1-SAPT4*), and secreted Saps play an important role in the spread of yeast in invasive infections, by facilitating tissue invasion and preventing the action of the host's phagocytic cells [15, 81, 92]. Negri *et al* 2010 [93] observed that C. *tropicalis* isolated from urine has a higher protease activity than blood and catheter isolates. Costa *et al* 2010b [85] in 112 isolates of *Candida* from oral mucosa, blood, and catheters detected 14 *C. tropicalis*, in which four from buccal mucosa and one from blood showed protease activity.

2.2 Phospholipases

The ability of microorganisms to invade host cells is related to damage to major cell membrane constituents, such as phospholipids [94]. *Candida* species use the secretion of phospholipases as an important virulence factor, because besides facilitating the tissue invasion, it assists in adherence and interferes in the host's defense mechanisms [15, 89, 95-97].

Phospholipases are a heterogeneous group of enzymes with the ability to hydrolyze one or more ester linkages in cell membrane glycerophospholipids. Phospholipases are classified as A, B, C, and D [94, 98]. These enzymes are expressed by seven classes of genes (*PLA*, *PLB1*, *PLB2*, *PLC1*, *PLC2*, *PLC3*, and *PLD1*). *PLB1* is considered as a virulence factor of *Candida* in *in vivo* studies about the pathogenesis of the disease, but the role of the other exoenzymes remains unknown. Plb1p is an 84 kDa glycoprotein, has hydrolase and lysophospholipase transacillase activity, and is present in yeast and pseudohypha cells during tissue invasion [10, 87, 94, 98].

Different levels of phospholipase activity among *Candida* species have been reported. In isolates from the oral cavity of individuals who use dental prostheses with or without stomatitis manifestation, Gümrü *et al* 2006 [96] and Marcos-Arias *et al* 2009 [99] found that *C. albicans* was the only species that showed phospholipase activity. Mohandas & Ballal 2011 [100] demonstrated that non-*albicans* species isolated from hospitalized patients in India were the main producers of phospholipase, with C. *guilliermondii, C. parapsilosis,* and *C. tropicalis* showing high enzymatic activity.

According to Treviño-Rangel *et al* 2013 [91] *C. orthopsilosis* was the species that presented most phospholipase activity among *C. parapsilosis* complex species, demonstrating that 69% of the isolates had very high enzymatic activity.

2.3 Extracellular lipases

Lipolytic enzymes are important in the infectious processes triggered by certain pathogenic fungi and are related to nutrient acquisition, adhesion to host tissues, synergistic interaction with other hydrolytic enzymes, nonspecific activation of inflammatory reaction, and self-defense, which are mediated by lysis of the competing microbiota [10, 89, 101]. Lipolytic enzymes catalyze cleavage and formation of ester linkages, which are the two major groups that are formed by lipases and esterases. The difference between these enzymes is their ability to act on soluble substrates.

Lipases act on a large amount of substrates, especially water-insoluble and long-chain, whereas esterases prefer substrates with lower hydrophobicity and triglycerides with fatty acids smaller than six carbons [87, 102, 103].

The lipases secreted by *C. albicans* are expressed by 10 genes (*LIP1 - LIP10*), and all types of lipase are observed during the transition from yeast to hyphae morphology [101]. *C. parapsilosis* has two genes coding for lipase secretion, CpLIP1 and CpLIP2, which participate in the pathogenesis of infections related to this species [15, 89]. Gácser *et al* 2007 [104] demonstrated that a lipase inhibitor (ebelactone B) significantly reduced tissue damage during *C.*

parapsilosis infection, and that biofilm formation was inhibited by CpLIP1/CpLIP2 mutants, thus confirming that isolates that do not exhibit functional lipases have less ability to develop infection in experimental models.

The activity of lipolytic enzymes is a target to investigate as a virulence factor of *Candida* spp, and several species are able to secrete these enzymes. Pakshir *et al* 2013 [23] showed that 87% and 47% of the isolates of *C. albicans* and *C. parapsilosis,* respectively, presented lipolytic activity. Among the species of the *C. parapsilosis* complex, Treviño-Rangel *et al* 2013 [91] reported that 67% of *C. orthopsilosis* isolates and 13% of *C. parapsilosis sensu stricto* had high lipolytic activity.

2.4 Hemolytic factor

The *Candida* species produces hemolytic factor in order to acquire iron by degrading hemoglobin, from lysis of erythrocytes. This important virulence factor allows survival and persistence of the pathogen in the host in order to establish the infection [15, 3, 77]

The hemolytic factor secreted by *C. albicans* is a cell wall mannoprotein, and the carbohydrate portion binds to the erythrocyte membrane promoting the lysis of these cells [105]. Although some cases indicate, *C. albicans* is the only species with hemolytic capacity, the secretion of the hemolytic factor has been observed in a variety of ways among clinical isolates of several *Candida* species [23, 106, 107]. Favero *et al* 2014 [108] demonstrated that *C. tropicalis* isolated from bloodstream infections has a higher hemolytic capacity than *C. albicans*. Hemolysis production has been verified in *C. parapsilosis* and *C. tropicalis* in different anatomical sites. *C. tropicalis* isolated from blood produces a greater halo of hemolysis than those from tracheal and skin secretions; however, *C. parapsilosis* isolates from tracheal secretion have a higher hemolytic capacity compared to those of blood [77].

2.5 Adherence

The ability to adhere to host cells and extracellular matrix as well as abiotic surfaces (intravascular and bladder catheters, cardiac valves, and dental prostheses) is an important feature of *Candida* species [78, 79, 83, 109, 110]. The adhesion mechanism that characterizes candidiasis during the infectious process triggers an endocytosis and active penetration of the yeasts into the cells, with tissue aggression and a greater capacity for dissemination by the host [83, 111].

The cell wall components of the *Candida* species determine the adhesion mechanisms of these fungi in the host cell. The cell wall of *C. albicans* has a variety of molecules that allow differentiation into two layers: external and internal [112]. The outer structure of the wall consists of glycoproteins, formed by covalent bonds of mannan and proteins (mannoproteins), whereas the inner layer contains polysaccharides of chitin and β-1,3-glycan that are responsible for a greater resistance and form of the fungal cell. Cell wall proteins (CWPs), which covalently bound to polysaccharide structures, are divided into two classes: CWPs bound to β-1,6-glycan by glycosylphosphatidylinositol (GPI) remnants and Pir (protein internal repeats) proteins that are directly bound to β -1,3-glycan, thus most adhesins on the cell surface of the *Candida* species are modified CWP-GPI [79, 113].

The action of the adhesin proteins in *C. albicans* is controlled by the expression of several genes, which are influenced by the morphology of the yeast and type of receptor in the host [112]. The *ALS* (agglutinin-like sequence) genes encode eight adhesin proteins (Als1-7 and Als9). However, Als3 is considered more important, because it is associated with the yeast filamentous phase, and it participates in the mechanism of endocytosis and acquisition of extracellular iron by the yeast [114].

Also with respect to *C. albicans*, adhesion Hwp1 (hyphal wall protein 1) expressed by the *HWP* gene, mediates adhesion to the epithelial cell, by functionally resembling the proteins of the host cell. Other adhesin proteins such as Iff11 and Hyr1 (hyphally upregulated protein 1) regulated by the *IFF* (*IPF* family *F*) and *HYR* genes are specific to hyphae, and are related to resistance to neutrophils [79, 83, 109, 114-116].

Candida non-*albicans* species express genes that encode adhesins; however, the mechanism of these adhesion proteins has not been fully elucidated. The *EPA* (epithelial adhesin) gene of *C. glabrata* participates in the adhesion of this yeast to epithelial cells [117], and the main adhesin, Epa1, is a calcium-dependent lecithin that binds to N-acetyl glycol conjugates which contain lactosamine from the host [118]. The adhesion of *C. parapsilosis* involves five proteins of the family Als and six Pga 30 (proteins anchored by glycosylphosphatidylinositol), and three Als are known to be involved in the adhesion processes of *C. tropicalis* [15, 78].

2.6 Biofilm formation

Biofilm formation by microorganisms is a protection mechanism for their development, because it helps evade the host's defense mechanisms, provides greater resistance to antifungal treatment, and enhances competitiveness with other infectious agents [15, 83, 119, 120]. The biofilm structure consists of a community of microorganisms, surrounded by an extracellular matrix, which develops both on host tissues and on hospital medical materials [10, 83].

Biofilm formation occurs through a sequential process that begins with adhesion to a substrate (adhesion step),

followed by synthesis of an extracellular matrix (maturation stage), and then dispersion of yeast around the substrate (dispersion step) [121].

The adhesion process of yeasts to the substrate consists primarily of nonspecific interactions, followed by a stronger adhesion by the adhesin proteins present in the fungal cell wall [122-125]. In this stage, the *BCR1* (biofilm and cell wall regulator 1) gene is a regulatory factor for the transcription of adhesin proteins in the yeast cell wall (*ALS1, ALS3, HWP1*) [126, 127]. The *ALS* family (Als 1 and Als 3) participates in the coaggregation of yeast cells, whereas the *HWP1* gene encodes adhesins present on the surface of hyphae [128]. This first step may also be influenced by genes responsible for adhesion on abiotic surfaces, such as *EAP1* (enhanced adherence to polystyrene) and *CSH1* (cell surface hydrophobicity 1), which is related to cell surface hydrophobicity [120].

The extracellular matrix maintains the integrity of the biofilm structure, due to its capacity to restrict entry of antifungal agents used to treat the disease [122, 129-131]. However, other associated characteristics, such as rate of cell growth, expression of genes that confer resistance, and presence of persistent cells that are able to escape antifungal agent and reconstitute the biofilm, determine a multifactorial phenomenon for developing resistance to antifungals [129, 131-133].

The dispersion step is characterized by the release of yeast cells from the biofilm structure, which may result in formation of new biofilms or propagation to other host tissues. At this stage, some characteristics observed include the reversal of hyphae to yeasts, new transcription regulators specific for this phase (Ume6, Pes1, and Nrg1 - dispersion regulators) which reduce or increase the release of cells, and a higher adhesion and filamentation capacity than planktonic cells [121].

During the formation of the *Candida* biofilm, an intercellular communication system, known as quorum sensing, which modulates its development, allows growth and dispersion of yeast cells [129, 134]. Some molecules of this communication system, such as tyrosol and farnesol, are important in biofilm formation. Tyrosol promotes the formation of hyphae and biomass in the initial phase of the biofilm. However, farnesol inhibits the formation of hyphae, preventing the growth of the biofilm and allowing the dispersion of the cells to other anatomical sites in the human host [120, 121, 125, 129, 135, 136].

The ability to form biofilm by *Candida* species has been extensively investigated in different casuistics. Pannanusorn *et al* 2013 [137] verified the ability of for biofilm formation in 393 *Candida* species that were isolated from bloodstream infections, and they found that *C. albicans* (40.3%) was less able to form biofilms than non-*albicans* species (88.7%). Among these species, 100% of the *C. tropicalis* and *C. lusitaniae* isolates formed biofilm, followed by 95% of *C. glabrata*, 86% of *C. dubliniensis*, and 67% of *C. parapsilosis*. The higher capacity to form biofilm of species in the *Candida* genus when compared to *C. albicans*, which is the most pathogenic species, was also reported by Mohandas & Ballal 2011 [100] and Ferreira *et al* 2013 [107] where they used isolates from different clinical and environmental samples. Tosun *et al* 2013 [89] demonstrated that only *C. parapsilosis sensu stricto* was able to form biofilm, whereas Lattif *et al* 2010 [138] found that all species of the *C. parapsilosis* complex were able to form biofilm.

The development of biofilm by different *Candida* species, according to the site of infection, was verified by Villar-Vidal *et al* 2011 [139], where they observed that *C. albicans* and *C. dubliniensis* isolated from blood present a higher metabolic activity in the formation of the biofilm compared to isolates of the buccal mucosa. However, Junqueira *et al* 2011 [140] found that the pathogenicity of *C. albicans* isolates from oral and systemic infections had the same biofilm formation capacity, regardless of the site of infection.

2.7 Switching phenomenon

The great morphological variability of *Candida* species is an important factor that determines their ability to establish and disseminate in new habitats [140, 141]. Hence, the switching phenomenon is a characteristic developed *in vitro* by the *Candida* species, with the purpose of assuming different macroscopic characteristics and that is associated to diverse changes at the cellular level [141, 142]. These phenotypic changes are reversible only in a small percentage of the cell population; however, the characteristic of the colony is steadily passed down for many generations after the initial event [141]. This allows these microorganisms to adapt rapidly to different microenvironments, promoting tissue invasion, evasion of the host's immune system, and antifungal resistance [141, 143].

According to Soll 2009 [144], the switching phenomenon verified in *C. albicans* isolates shows a white-opaque change system. Thus, when cells from a single white colony are inoculated in nutrient-poor media, most of the colonies formed exhibit the white phenotype, but a minority ($\sim 10^{-3}$) exhibits the opaque phenotype. When cells from a single opaque colony are inoculated at a low density, most of the colonies that form are opaque and a minority is white.

Accentuated differences are observed between white and opaque cells in relation to the cellular phenotype: the white cells are round to oval with a smooth surface, while the opaque cells are twice as large as the previous ones and have a rough surface. Some researchers have shown that opaque cells are less virulent than white cells in experimental models of systemic infections in animals [98, 141, 145].

In *C. parapsilosis,* four phenotypes are observed that characterize the switching phenomenon: rough, concentric, crateriform, and smooth colonies. Cells from rough and concentric colonies are predominantly formed by pseudohyphae, while cells from crateriform and smooth colonies are round. In polystyrene surfaces, the concentric phenotype has up to twice the capacity to form biofilm as the rugose, smooth, and crateriform colonies [146].

The evaluation of the switching phenomenon in *C. tropicalis* reveals four different types of colony phenotypes: annular, rugose, smooth, and semi-smooth. According to these characteristics, the biofilm formation occurs more intensely in phenotypes that present a greater amount of pseudohyphae (annular, rough, and semi-smooth). The presence of extracellular matrix during the development of the ring and rough phenotypes is correlated with the complex architecture of the colonies, which demonstrates a possible role of the extracellular matrix in the switching phenomena in *C. tropicalis* [76].

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