CELL SURFACE HYDROPHOBICITY AND BIOFILM FORMATION OF CANDIDA YEAST SPECIES IN DIFFERENT CULTURE MEDIA

ABSTRACT: Cell surface adhesion is considered an essential step in the spread, infection and persistence of Candida yeasts in the host. Their ability to adhere on biotic and abiotic surfaces depends on several factors, including hydrophobicity. Once attached, these yeasts are capable of growing in biofilms, which are constituted of structured communities of encapsulated cells within an extracellular matrix, resistant to antifungal agents. In this context, this study aimed to analyze the cell surface hydrophobicity and specific biofilm formation of six Candida strains in different culture media: Sabouraud dextrose broth (SDB), artificial saliva (AS), Roswell Park Memorial Institute medium (RPMI 1640) and N-acetylglucosamine-yeast nitrogen base proline (NYP). Six yeasts of the genus Candida were studied: three C. albicans (Ca): Ca ATCC 10231 and the clinical isolates Ca34 and Ca05 and C. parapsilosis (Cr): Cp ATCC 22019 and the clinical isolates Cp120 and Cp38. Hydrophobicity was calculated as the optical density due to the retention of the hydrophobic cells in hydrocarbon by the hydrocarbon-water biphasic assay following the MATH (Microbial Adhesion to Hydrocarbon) method. The biofilm formation index was calculated as the optical density obtained by the growth of the yeasts in the culture media in a polystyrene microtiter plate, subsequently stained with 1% violet crystal. The results showed that hydrophobicity varied according to the media and the yeasts studied, and two of these (Ca34 and Ca10231) presented significant variation between the media. A more hydrophobic character was observed in yeasts grown on RPMI 1640 medium, and those grown on Sabouraud dextrose broth appeared more hydrophilic. The specific biofilm formation index was more intense for RPMI 1640 than in other media, which was expected, due to its ability to induce the transition between yeast-hyphal morphology, which is one of the key factors involved in the adhesion of C. albicans on different surfaces. RPMI 1640 was the best medium for obtaining biofilm in vitro, due to its greater hydrophobicity, which can enhance cell adhesion to the polystyrene plate, and due to its nutrient content, necessary for complete cell growth and biofilm formation.


INTRODUCTION

Candida species are able to colonize biotic surfaces by the commensal pathway, especially in immunocompromised patients, and this may cause serious complications in their clinical condition (SARDI et al., 2013). Invasive candidiasis can be caused by different species such as Candida glabrata, Candida krusei, Candida dubliniensis, Candida parapsilosis and Candida tropicalis, with most the prevalent cases reported for Candida albicans (SILVA, 2011; CORNELY et al., 2012).

The ability of Candida yeasts to infect diverse host niches is supported by a wide range of virulence factors and fitness attributes. Among the virulence factors, morphological transition between yeast and hyphal forms, the expression of adhesins and invasins on the cell surface, thigmotropism, the formation of biofilms, phenotypic switching and the secretion of hydrolytic enzymes stand out (ACHKAR; FRIES, 2010; SILVA et al., 2011, MAYER et al., 2013). Rapid adaptation to changes in environmental pH, metabolic flexibility, powerful nutrient acquisition systems and robust stress response machinery are defined as fitness attributes (MAYER et al., 2013; BROWN et al., 2014).

The first factor involved in the colonization of human tissues by Candida is the adhesion of yeast cells to a supporting surface, followed by the formation of surface-associated microbial communities called biofilms (BACHMAN et al., 2002). After adhesion of yeast cells, the process of biofilm formation is followed by cell proliferation that forms a basal layer along the surface, and the biofilm is enclosed by secreted extracellular matrix materials, forming complex three-dimensional architecture (HSUEH-FEN, CHUNG-YU, 2015).

Candida albicans can form biofilms on inert surfaces, such as a variety of indwelling medical devices, most notably intravenous catheters and...
intrauterine devices (ACHKAR; FRIES, 2010). Biofilm provides a safe haven for fungal cells and serves as a reservoir for sources of infection, and this has negative consequences as it leads to high levels of resistance to most clinically used antifungal agents. During this mode of growth, Candida resists the normal immune response, often causing fungal agents. During this mode of growth, Candida resists the normal immune response, often causing devastating disease (GHANNOUM et al., 2015; PIERSE et al., 2015).

In this context, the analysis of hydrophobicity and biofilm formation of Candida species provides important information about the relationship between adhesion and growth of biofilm-associated cells on different surfaces. The present study aimed to evaluate the hydrophobicity of cell surface in Candida species calculate Specific Biofilm Formation Indexes and make the interconnection between hydrophobicity and Specific Biofilm Formation of Candida yeast, when grown in different culture media: Sabouraud dextrose broth (SDB), artificial saliva (AS), Roswell Park Memorial Institute medium (RPMI-1640) and N-acetylglucosamine-yeast nitrogen base-proline (NYP).

MATERIAL AND METHODS

This study was performed using six yeasts of the genus Candida. These were three C. albicans (Ca): Ca ATCC 10231 and the clinical isolates Ca34 and Ca05; and three C. parapsilosis (Cp): Cp ATCC 22019 and the clinical isolates Cp120 and Cp38. The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouraud dextrose agar (Sigma-Aldrich, MO, USA). The strains were from the collection of the Laboratory of Mycology of the Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, Brazil. Yeast cultures were maintained as frozen stocks (-20 °C) in Brain Heart Infusion broth (BHI) with 20% glycerol (Sigma-Aldrich, MO, USA). The cells were reactivated on Sabouradu
turbidity remaining in this phase after addition of hydrocarbon was used.

All assays were performed in three independent experiments, and the results were statistically analyzed by using analysis of variance (ANOVA) and Tukey test (p < 0.05).

RESULTS AND DISCUSSION

Significant differences were observed in biofilm formation of Candida yeast in the different culture media, suggesting an important influence from the medium on the ability of strains to express this virulence factor (Tables 1 and 2).

Table 1. Specific Biofilm Formation index on different culture media.

<table>
<thead>
<tr>
<th>Candida</th>
<th>SDB Mean ± SD</th>
<th>AS Mean ± SD</th>
<th>RPMI-1640 Mean ± SD</th>
<th>NYP Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca05</td>
<td>0.030 ± 0.011</td>
<td>0.167 ± 0.123</td>
<td>3.195 ± 0.057</td>
<td>0.273 ± 0.003</td>
</tr>
<tr>
<td>CaATCC10231</td>
<td>0.041 ± 0.037</td>
<td>0.063 ± 0.013</td>
<td>1.524 ± 0.291</td>
<td>0.224 ± 0.059</td>
</tr>
<tr>
<td>Ca34</td>
<td>0.019 ± 0.021</td>
<td>0.251 ± 0.270</td>
<td>3.519 ± 0.685</td>
<td>0.331 ± 0.012</td>
</tr>
<tr>
<td>CpATCC22019</td>
<td>0.384 ± 0.150</td>
<td>0.225 ± 0.010</td>
<td>0.542 ± 0.106</td>
<td>0.056 ± 0.033</td>
</tr>
<tr>
<td>Cp38</td>
<td>0.924 ± 0.548</td>
<td>0.905 ± 0.149</td>
<td>2.259 ± 1.007</td>
<td>0.265 ± 0.147</td>
</tr>
<tr>
<td>Cp120</td>
<td>0.099 ± 0.030</td>
<td>0.201 ± 0.111</td>
<td>0.605 ± 0.228</td>
<td>0.032 ± 0.019</td>
</tr>
</tbody>
</table>

SDB - Sabouraud dextrose broth, AS - artificial saliva, RPMI-1640 - Roswell Park Memorial Institute medium 1640, NYP - N-acetylglucosamine-yeast nitrogen base-proline, Ca - Candida albicans and Cp - Candida parapsilosis, SD - standard deviation.

Table 2. Analysis of variance (two-way ANOVA) for Specific Biofilm Formation index.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
<th>F ratio (F statistic)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor A (Strains)</td>
<td>1.5036</td>
<td>5</td>
<td>0.3007</td>
<td>1.9357</td>
<td>0.1447</td>
</tr>
<tr>
<td>Factor B (culture media)</td>
<td>5.7715</td>
<td>3</td>
<td>1.9238</td>
<td>12.3837</td>
<td>0.0002</td>
</tr>
<tr>
<td>Interaction AB</td>
<td>2.3303</td>
<td>15</td>
<td>0.1554</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9.6054</td>
<td>23</td>
<td>0.4176</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RPMI-1640 medium provided the highest biofilm formation indexes. This culture medium has a high nutrient content, which favors the growth of planktonic cells. Its formulation simulates the composition of human fluids with 0.45% glucose, 6 g.L⁻¹ phosphate, amino acids and biotin (CHANDRA et al., 2001).

RPMI 1640 medium is considered to induce the yeast-to-hypha transition, which is one of the key factors involved in the adhesion of C. albicans on different surfaces (BLANKENSHIP; MITCHELL, 2006). Furthermore, morphological changes between yeast to hyphae and pseudohyphae promote host tissue colonization, as a primary manifestation of invasion and the beginning of pathogenesis (KUMAR et al., 2000).

NYP medium has all the vitamins and minerals needed for yeast cultivation, except amino acids, which are important sources of nitrogen and carbohydrate. The addition of N-acetylglucosamine and proline to the NYP medium was proposed by Marichal et al. (1986), with the objective of promoting yeast filamentation, since both have an influence on the transitions between yeast and hyphal forms (ODDS, 1988; CALDERONE, 2001). Candida species also have the ability to use N-acetylglucosamine as the sole carbon source (SINGH; BISWAS; DATTA, 1980).

The SDB showed the highest readings for yeast total growth. However, its performance in biofilm formation was weak. The peptones and dextrose in SDB composition have the purpose of promoting fungal growth. Peptones provide nitrogen, vitamins, minerals, amino acids and growth factors, while dextrose is the energy source for the growth of microorganisms. The low pH favors fungal growth and inhibits bacterial contamination.

Acidity is inhibitory to a large number of bacteria, making the medium particularly useful for the cultivation of fungi and "low-pH" microorganisms (EMMONS; BINFORD; UTZ, 1970; SABOURAUD, 1892).
Cardoso (2004) reported that the artificial saliva medium provided a greater production of extracellular matrix in *Candida* while the RPMI-1640 medium provided a high level of metabolic activity. It was noted that biofilm formation was strongly influenced by culture medium. On the other hand, the analyzed yeasts did not interfere in the results achieved (p < 0.005). However, in our study, the artificial saliva culture medium provided slight biofilm formation results. The observed differences were significant only when compared to the results obtained by the RPMI 1640, and all readings were lower in the artificial saliva medium.

The significant differences between RPMI 1640 and the other culture media are shown in Table 3. However, significant differences between SDB, AS and NYP were not detected for biofilm formation.

### Table 3. Tukey's Honestly Significant Difference (HSD) test among the averages of biofilm formation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean difference</th>
<th>p-value</th>
<th>Significance</th>
<th>Accepted hypothesis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDB vs. NYP</td>
<td>0.0922</td>
<td>0.5728</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>SDB vs. RPMI-1640</td>
<td>-1.1093</td>
<td>0.0048</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>SDB vs. AS</td>
<td>-0.0387</td>
<td>0.9884</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>NYP vs. RPMI-1640</td>
<td>-1.2015</td>
<td>0.0005</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>NYP vs. AS</td>
<td>-0.1308</td>
<td>0.9381</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>RPMI-1640 vs. AS</td>
<td>1.0707</td>
<td>6</td>
<td>yes</td>
<td></td>
</tr>
</tbody>
</table>

SDB - Sabouraud dextrose broth, AS - artificial saliva, RPMI 1640 - Roswell Park Memorial Institute medium 1640, NYP - N-acetylglucosamine-yeast nitrogen base-proline.

The data showed an honestly significant variation between the values of SCH in the culture media for the yeasts Ca34 and Ca10231, indicating the dependence of hydrophobicity on the growth media for these strains. However, the same observation could not be made for the remaining strains, since the hydrophobicity remained uniform with variation of the media.

Thus, these results allow us to affirm that although differences between the strains analyzed were not significant, the employed culture media modulate both the formation of biofilm phenomena and the cell hydrophobicity of yeasts, and there was a correlation between these two characteristics.

According to Acosta (2009), different degrees of cell surface hydrophobicity observed in *Candida* species in different culture media.

### Table 4. Percentage of cell surface hydrophobicity obtained for *Candida* species in different culture media.

<table>
<thead>
<tr>
<th>Candida</th>
<th>SDB Mean ± SD</th>
<th>AS Mean ± SD</th>
<th>RPMI-1640 Mean ± SD</th>
<th>NYP Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca05</td>
<td>61.58 ± 3.04</td>
<td>26.55 ± 0.36</td>
<td>52.07 ± 7.06</td>
<td>53.94 ± 6.55</td>
</tr>
<tr>
<td>Ca10231</td>
<td>8.32 ± 0.60</td>
<td>38.45 ± 1.52</td>
<td>31.51 ± 1.38</td>
<td>48.21 ± 0.92</td>
</tr>
<tr>
<td>Ca34</td>
<td>0.99 ± 0.58</td>
<td>25.12 ± 5.73</td>
<td>74.75 ± 1.04</td>
<td>50.36 ± 4.06</td>
</tr>
<tr>
<td>Cp22019</td>
<td>22.30 ± 1.07</td>
<td>23.72 ± 3.19</td>
<td>30.14 ± 1.68</td>
<td>21.48 ± 7.67</td>
</tr>
<tr>
<td>Cp38</td>
<td>76.83 ± 1.43</td>
<td>70.42 ± 5.21</td>
<td>81.18 ± 1.39</td>
<td>58.12 ± 2.07</td>
</tr>
<tr>
<td>Cp120</td>
<td>10.89 ± 1.80</td>
<td>23.70 ± 1.19</td>
<td>29.04 ± 5.92</td>
<td>13.55 ± 1.92</td>
</tr>
</tbody>
</table>


The highest hydrophobicity was observed for the strain Cp38, with a CSH average of 71.64%, in the tested media. The Cp120 yeast showed the lowest CSH average, with only 19.30%. Regarding culture media, RPMI 1640 showed the highest CSH average (49.78%), followed by NYP (40.94%), AS (34.66%), and SDB (30.15%).

In studies about cell hydrophobicity, Blanco et al. (2010) observed that RPMI 1640 medium promotes the development of a hydrophobic character in *C. albicans* species, which is variable among strains, and that yeasts growing in Sabouraud medium developed a hydrophilic character. Thus, yeasts are more likely to produce biofilm in RPMI 1640 than in SDB.
distinct Candida species may be related to a greater or lesser capacity of the yeasts to adhere onto various surfaces.

C. tropicalis, C. glabrata and C. krusei are highly hydrophobic and have the capacity to adhere to a large number of polymeric surfaces, when compared to less hydrophobic species, such as C. albicans, C. parapsilosis and C. stellatoidea. The adhesion capacity to different cells is a key factor for the dissemination and persistence of yeast infection in body tissues. Among other factors, adhesion depends on the nature and type of surface, the environment, surface shape, the homogeneity of the medium, the presence or absence of surface charges, electrolyte concentrations, hydrophobicity, hydrodynamic characteristics, the concentration and nutrient distribution on the surface, suitable pH and temperature (MARSHALL, 1994).

This study showed that both biofilm formation and cell surface hydrophobicity are strongly influenced by the culture media employed. Specifically, the RPMI 1640 medium was the most favorable to biofilm growth in yeasts, indicating that environmental conditions, such as the availability of nutrients, pH and amount of oxygen may affect the formation and composition of biofilms (FINKEL; MITCHELL, 2011; SARDI et al., 2013).

According to Sardi et al. (2013), the process of biofilm formation is a phenomenon conducted and regulated by various cellular signaling cascades between the fungus and the environment. The initial adhesion is mediated by non-specific factors, such as hydrophobicity and electrostatic forces. This process is followed by specific interactions, promoted by adhesins present on the surface of fungal cells, which bind to amino acids and sugars present on the host cell surface or which promote adhesion to abiotic surfaces.

Cell surface hydrophobicity was variable among the strains, but the yeasts were generally more hydrophobic in RPMI 1640 medium and more hydrophilic in Sabouraud dextrose broth. This difference was not significant enough to make a correlation between hydrophobicity and biofilm formation.

Finally, this study contributes to further research about the impact of different environmental conditions on cell surface hydrophobicity, biofilm formation and the susceptibility of these properties to different antifungals. Furthermore, it contributes to developing a standardized methodology for these purposes.

ACKNOWLEDGMENTS

We are grateful to the National Council for Scientific and Technological Development (CNPq) for financial assistance Nº 475935 / 2011-0; to the State University of Goiás (UEG) for the Incentive Scholarship Program for Research and Scientific Production (PROBIP-UEG); the Coordination for the Improvement of Higher Education Personnel (CAPES) for scholarships in Scientific Initiation and Master’s degree; and the Research Support Foundation of the State of Goiás (FAPEG) for supporting participation in scientific events (public Call 01/2015).

RESUMO: A adesão é considerada um passo essencial para a disseminação, infecção e persistência das leveduras do gênero Candida nos hospedeiros. A habilidade dessas leveduras em se aderir a superfícies bióticas e abióticas depende de fatores, incluindo a hidrofobicidade da superfície celular. Uma vez aderidas, estas leveduras são capazes de crescer como biofilmes, os quais são caracterizados como comunidades estruturadas de células encapsuladas dentro de uma matriz extracelular, resistentes a drogas antifúngicas. Neste contexto, objetivou-se analisar a hidrofobicidade da superfície celular e a formação específica de biofilme de Candida spp. em diferentes meios de cultura: caldo Sabouraud dextrose (CSD), saliva artificial (SA), Roswell Park Memorial Institute medium (RPMI 1640) e N-acetylglicosamin-yeast nitrogen base-proline (NYP). Foram estudadas seis leveduras do gênero Candida, sendo três C. albicans (Ca): Ca ATCC 10231 e os isolados clínicos Ca34 e Ca05 e C. parapsilosis (Cp): Cp ATCC 22019 e os isolados clínicos Cp120 e Cp38. A hidrofobicidade foi calculada como a porcentagem da redução da turbidez da fase aquosa, devido a retenção das células hidrofóbicas no hidrocarboneto pelo método bifásico água-hidrocarboneto MATH (Teste de Adesão Microbiana a Hidrocarbonetos). O índice de formação de biofilme foi calculado com as densidades ópticas obtidas por meio do crescimento das leveduras nos meios de cultura em poços de placa de microtitulação de polietileno e posteriormente corados com cristal violeta a 1%. Os resultados demonstraram que a hidrofobicidade variou de acordo com os meios e as leveduras estudadas, sendo que duas (Ca34 e Ca10231) apresentaram variação significativa entre os meios. Foi verificado um caráter mais hidrofóbico das células crescentes em RPMI 1640 e mais hidrofílico nas células crescidas em caldo Sabouraud dextrose. A formação específica de biofilme apresentou-se mais intensa em RPMI 1640 do que nos outros meios, o que já era esperado pela sua capacidade de induzir o processo de transição levedura-hifas, o qual é considerado um dos fatores cruciais envolvidos na adesão de leveduras. Com os resultados obtidos, infere-se que o RPMI 1640 é o melhor meio para obtenção de biofilme in vitro, pois as células foram mais hidrofóbicas, o que pode aumentar a adesão das
mesmas à placa de poliestireno, e oferecer nutrientes necessários para o completo desenvolvimento das células e do próprio biofilme.

**PALAVRAS-CHAVE:** Biofilme. Hidrofobicidade. Candida albicans. Candida parapsilosis

**REFERENCES**


Cell surface hydrophobicity…


