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Short Communication

In vitro evaluation of hydrolytic enzyme activity and biofilm formation of *Candida parapsilosis* species complex from a nosocomial environment

***Shaiana Paula-Mattiello*[1], *Sílvia Dias de Oliveira*[1] *and Renata Medina-Silva*[1]**

[1]. Faculdade de Biociências, Laboratório de Imunologia e Microbiologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brasil.

Abstract

Introduction: *Candida parapsilosis* complex species, frequently found in hospital environments, have gained importance as etiological agents of candidemia. **Methods:** *Candida parapsilosis* complex isolates from a nosocomial environment were identified and their hydrolitic enzyme activity and ability to form biofilm were characterized. **Results:** Twenty-two *C. parapsilosis sensu stricto* isolates produced proteinase and three produced phospholipase. Most *Candida metapsilosis* isolates produced proteinase and one also produced phospholipase. All 29 isolates formed biofilms. **Conclusions:** The nosocomial environment may act as a reservoir for *C. parapsilosis* complex isolates with phenotypic features that could possibly lead to nosocomial infections and health complications in hospital patients.

Keywords: *Candida parapsilosis* complex. Biofilm. Hydrolytic enzymes.

Candida species are major agents involved in nosocomial fungal infections worldwide, especially infections of endogenous origin and those related to immunocompromised patients[1]. Different factors in the nosocomial setting and the hands of healthcare workers may represent important sources for *Candida* infections[2]. *Candida parapsilosis* complex species (*Candida parapsilosis sensu stricto*, *Candida orthopsilosis*, and *Candida metapsilosis*) are often isolated from nosocomial environments; since the 1990s, they have gained importance as etiological agents of candidemia in hospitals in different countries[1].

The pathogenicity of *Candida* species may be at least partly due to their ability to produce hydrolytic enzymes, such as phospholipases (PLs) and aspartyl proteinases (SAPs), which are considered important factors for *C. parapsilosis* adherence, tissue penetration, and host invasion[3]. Moreover, the ability to form biofilms can induce a significant reduction in yeast antifungal susceptibility and an increase in their capacity to evade the immune system[4].

Most data regarding the ability to produce hydrolytic enzymes and biofilm formation has been obtained using clinical *C. albicans* isolates[1,5]; to date, few studies have investigated these properties in *C. parapsilosis* complex isolates, particularly those from nosocomial environments. In this context, the present study evaluated the ability of 29 *C. parapsilosis* complex isolates present in hospital settings to produce hydrolytic enzymes and form biofilms. These isolates were collected between December 2009 and February 2010, strictly from the nosocomial environment and medical devices in a general patient care unit at a tertiary care teaching hospital in Porto Alegre (RS-Brazil). Following the collections, the isolated yeasts were biochemically identified as belonging to the *C. parapsilosis* complex (data not shown).

For this study, yeast genomic deoxyribonucleic acid (DNA) was extracted as previously described[6] and used as template for amplification of a *FKS1* gene fragment (1,032bp). Amplicons were digested with *Eco*RI[7], separated by electrophoresis on a 2% agarose gel stained with ethidium bromide (0.5µg/mL), and analyzed using a Gel Doc L-Pix Image System (Loccus Biotecnologia, SP, Brazil). Fragments of 1,032bp indicated *C. parapsilosis sensu stricto*; fragments of 564 and 474bp indicated *C. metapsilosis*; and fragments of 474bp, 306bp, and 258bp indicated *C. orthopsilosis*. *C. albicans* ATCC 1884 was used as the negative control and *C. parapsilosis* ATCC 22019, *C. metapsilosis* ATCC 96143, and *C. orthopsilosis* ATCC 96141 were employed as positive controls.

Phospholipase and aspartyl proteinase production was analyzed and interpreted as previously described[8,9]. The average *Pz* values – the ratio between the colony diameter (*dc*) and *dc* plus precipitation zone (*dcp*) – were calculated and the isolates were grouped into five categories according to their *Pz* values[9].

Corresponding author: Msc. Shaiana Paula-Mattiello.
e-mail: shaiana_m@hotmail.com
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All biofilm assays were conducted 10 times for each isolate[10]. The isolates were classified into four categories: non-producers, weak, moderate, or strong biofilm producers[4]. Statistical comparisons regarding biofilm formation were performed employing the Student's *t* test with p-value ≤ 0.05, using the software Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM, USA), available at PUCRS.

All isolates were confirmed by *FKS1* gene amplification as belonging to the *C. parapsilosis* complex (**Table 1**); 22 (75.9 %) were identified as *C. parapsilosis sensu stricto*, seven (24.1%) as *C. metapsilosis*, and none as *C. orthopsilosis*. The predominance of *C. parapsilosis sensu stricto* among the species from this complex has previously been observed in clinical and environmental samples[11,12]. Conversely, *C. metapsilosis* and

TABLE 1

Species identification, original collection site, and proteinase and phospholipase activities of nosocomial *Candida parapsilosis* complex isolates.

Isolates	Origin	Proteinase activity (*Pz*)	Rating	Phospholipase activity (*Pz*)	Rating
CP.01	Chair 1	0.40	+ + + +	1.0	-
CP.02	Chair 2	0.31	+ + + +	1.0	-
CP.03	Bed 4	0.46	+ + + +	0.55	+ + + +
CM.04	Medication trolley 2	0.54	+ + + +	1.0	-
CP.05	Sphygmomanometer 2	0.47	+ + + +	1.0	-
CP.06	Sphygmomanometer 3	0.36	+ + + +	1.0	-
CP.07	Glove 1	0.35	+ + + +	0.52	+ + + +
CP.08	Room table 1	0.55	+ + + +	1.0	-
CM.09	Sphygmomanometer 4	1.0	-	1.0	-
CP.10	Room table 2	0.28	+ + + +	0.60	+ + + +
CP.11	Medication table 1	0.39	+ + + +	1.0	-
CP.12	Sphygmomanometer 1	0.43	+ + + +	1.0	-
CP.13	Glove 2	0.42	+ + + +	1.0	-
CP.14	Wheelchair 1	0.38	+ + + +	1.0	-
CP.15	Bed 5	0.52	+ + + +	1.0	-
CP.16	Sphygmomanometer 4	0.47	+ + + +	1.0	-
CP.17	Feed table 1	0.40	+ + + +	1.0	-
CP.18	Medication trolley 1	0.37	+ + + +	1.0	-
CM.19	Sphygmomanometer 3	0.57	+ + + +	1.0	-
CP.20	Feed table 2	0.40	+ + + +	1.0	-
CP.21	Medication trolley 2	0.40	+ + + +	1.0	-
CP.22	Medication trolley 3	0.47	+ + + +	1.0	-
CM.23	Medication trolley 4	0.55	+ + + +	1.0	-
CP.24	Medication trolley 5	0.36	+ + + +	1.0	-
CM.25	Sphygmomanometer 5	0.38	+ + + +	1.0	-
CP.26	Feed table 3	0.37	+ + + +	1.0	-
CM.27	Bed 1	1.0	-	1.0	-
CM.28	Feed table 1	0.60	+ + + +	0.42	+ + + +
CP.29	Feed table 4	0.36	+ + + +	1.0	-
C. parapsilosis ATCC 22019	-	1.0	-	1.0	-
C. albicans ATCC 18804	-	0.95	+	0.87	++

CP: *Candida parapsilosis sensu stricto*; **CM:** *Candida metapsilosis*; ***C*:** *Candida;* ***Pz* = 1.0 (-):** no activity; ***Pz* 0.90-0.99 (+):** weak activity; ***Pz* 0.80-0.89 (+ +):** mild activity; ***Pz* 0.70-0.79 (+ + +):** strong activity; ***Pz* < 0.69 (+ + + +):** very strong activity.

C. orthopsilosis are not usually found or occur at very low frequencies in nosocomial environments; previous reports have suggested they are associated mainly with clinical samples and that their relative frequency varies among hospitals and geographic regions[11,12].

The ability of the isolates to produce proteinase and PL is detailed in **Table 1**. Our results indicate that both *C. parapsilosis sensu stricto* and *C. metapsilosis* produce mainly proteinase; most (93.1%) isolates were classified as very strong producers. The isolates also exhibited PL production; however, at a lower frequency (13.7%). Specifically, all 22 *C. parapsilosis sensu stricto* isolates demonstrated proteinase production, but only three showed PL activity. These data are similar to studies indicating that this species is a frequent strong proteinase producer, but often a non-PL producer[3,11]. Of the seven *C. metapsilosis* isolates, five produced proteinase, and interestingly, one was found to produce both enzymes. Phospholipase production is not usual for this species; the absence of PL activity in *C. metapsilosis* isolates has been previously described[13]. Although the role of PL in *Candida* virulence remains unclear, some authors recognize the secretion of proteinases as an important virulence factor, which is immunogenic during infection and able to promote the degradation of host defense proteins[3].

The development of the biofilm has been described as an important virulence factor for *Candida*[14]. In this context, the development of biofilm by *C. parapsilosis* complex has gained considerable attention because isolates from these species have been observed in an extensive variety of biotic and abiotic surfaces[14]. In our study, all 29 isolates formed biofilms; 12 were classified as weak and 15 as moderate biofilm producers (**Figure 1**). Only two isolates were strong biofilm producers and both were identified as *C. parapsilosis sensu stricto*. Furthermore, *C. parapsilosis* ATCC 22019 and *C. albicans* ATCC 18804 were classified as moderate and weak biofilm producers, respectively. No significant difference in biofilm production was detected between *Candida* species (p-value=0.71). However, over half of the isolates (51.7%) were characterized as strong or moderate producers. This indicates a significant frequency of potentially pathogenic *Candida* species that can adhere efficiently to surfaces and tissues, representing an important risk when occurring in nosocomial environments.

The data presented in this study revealed that all *Candida* isolates investigated were able to form biofilms and most also exhibited the ability to efficiently produce proteinase. These findings emphasize the potential pathogenicity of nosocomial *C. parapsilosis* complex isolates from hospital environment origins because they demonstrate the maintenance of important virulence factors when these yeast species leave host tissues[15], which may represent a potential risk for reinfection of hospitalized patients. Thus, our results contribute to the characterization of the clinical risk posed by *C. parapsilosis sensu stricto* and *C. metapsilosis* isolates occurring in nosocomial environments and provide pertinent information regarding the behavior of pathogenic microorganisms in hospital settings. These findings may be used in the design and application of daily disinfection and antisepsis practices for healthcare workers.

Our findings improve information concerning phenotypic properties of clinically important yeast species that efficiently contaminate surfaces and objects in hospital settings. Thus, they are relevant for the understanding of both sporadic cases and outbreaks of invasive fungal infections in hospitals, especially in immunocompromised patients. Further analyses, aiming measure the levels of hydrolytic enzymes expression, as well

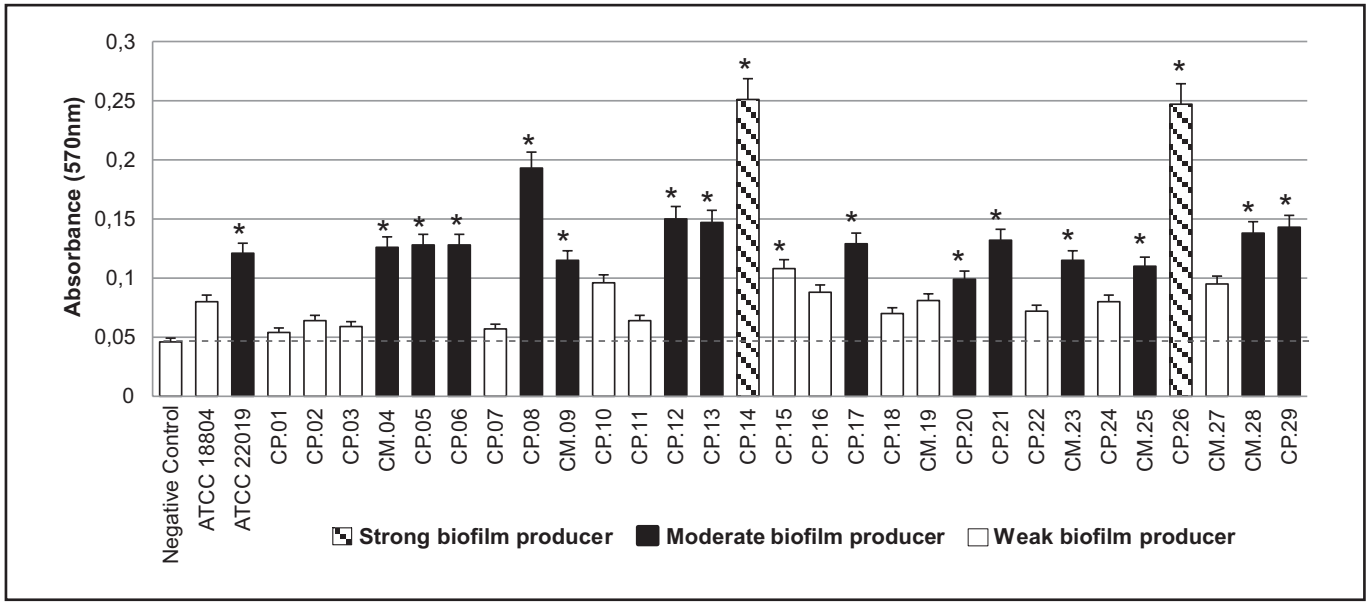

FIGURE 1 – Biofilm production by *Candida parapsilosis* complex isolates. The biofilm formation of all 29 isolates on polystyrene plates was quantified by measuring absorbance (A_{570nm}). **CP:** *Candida parapsilosis sensu stricto*; **CM:** *Candida metapsilosis*; **ATCC 22019:** *Candida parapsilosis*; **ATCC 18804:** *Candida albicans*. The error bars represent the variation of 10 replicates. The dashed line indicates the cut-off for biofilm classification (A_{570nm} = 0.048).

as to evaluate other virulence factors - like the mechanisms of antimicrobial resistance of the isolates in the biofilm condition - would importantly contribute to enhance the characterization of yeast species of the *C. parapsilosis* complex occurring in the nosocomial environment.

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Conflict of interest

The authors declare that have no conflicts of interest.

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