

**Title: PRODUCTION OF ASPARTIC PROTEASE (SAP) AMONG CLINICAL AND ENVIRONMENTAL STRAINS OF *Candida albicans* AND *Candida non-albicans* SUBMITTED TO SUBINHIBITORY CONCENTRATIONS OF ANTIFUNGAL AGENTS**

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**Abstract:**

*Candida* spp. have become, in recent decades, important causing agents of invasive infections and these are responsible for highest levels of morbidity and mortality. It is believed that secreted aspartic protease (Sap) is a factor directly associated to the infectious process and plays key role in the pathogenicity of *Candida* spp. and its production may be modulated by the exposure to subinhibitory concentrations of antifungal agents. 100% of the isolates were susceptible to Amphotericin B, Voriconazole and Caspofungin and 89,9% to Fluconazole. 7,4% of the isolates showed dose-dependent susceptibility and 3,7% showed resistance to Fluconazole. In the qualitative analysis of proteolytic activity, 77,7% of the isolates showed activity and in the quantitative analysis all they showed to be active. The highest level of activity was observed in *Candida* complex "*psilosis*" 210 (100%) and the lowest in *C. albicans* 257 (2,44%). The most of the isolates of *C. albicans* (50%) was classified as weakly proteolytic, while *Candida non-albicans* (53%) as moderately proteolytic. The presence of antifungals in the cultivation changed significantly the percentage of proteolytic activity of most of the isolates. The highest difference of percentage was observed in *C. lusitanae* 286, which in the presence of  $\frac{1}{4}$  of IC<sub>90</sub> of amphotericin B showed increase of 13,7x in relation to the absence of this drug. From the quantitative analysis of purified Sap by molecular exclusion chromatography was observed high proteolytic activity in samples that had low protein levels. This shows that the proteolytic activity may not be associated to high level of secreted proteins, but mainly to the proteolytic capability of the enzyme. The metabolic activity of most of the isolates was significantly changed in the presence of subinhibitory concentrations of antifungals. The highest difference of percentage was observed in *Candida* complex "*psilosis*" 210, who in the presence of  $\frac{1}{2}$  of IC<sub>50</sub> of fluconazole showed reduction at 8x in the metabolic activity in relation to the absence of this drug. There was SAP2 expression only in *C. albicans* ATCC 64548 and this was significantly reduced in the presence de  $\frac{1}{4}$  of IC<sub>90</sub> of amphotericin B. The Sap activity may be considered a potential factor associated to the virulence, once that highest proteolytic activity was observed in isolate with reduced susceptibility to antifungals.

**Keywords:** Virulence factors, *Candida albicans*, Sap, Antifungals.

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