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

Authors: Silva, N.C.¹, Oliver, J.C.¹, Nery, J.M.¹, Ariosa, M.C.F.¹, Chavasco, J.K.¹, Paula, C.R.², Brigagão, M.R.P.L.¹, Dias, A.L.T.¹

Institution: ¹ICB (Instituto de Ciências Biomédicas), Universidade Federal de Alfenas (Rua Gabriel Monteiro da Silva, 700, 37130-000, centro, Alfenas-MG), ²Faculdade de Odontologia da Universidade de São Paulo (Av. Professor Lineu Prestes, 2227, 05508000, Butantã, São Paulo)

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 actives. 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. lusitaniae 286, which in the presence of ½ of IC₅₀ 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 ½ of IC₉₀ 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 ¼ of IC₉₀ 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.

Funding agency: Fundação de Amparo à pesquisa do Estado de Minas Gerais (FAPEMIG)