

PRODUCTION OF SECRETED ASPARTYL PROTEINASES BY *CANDIDA* sp. CLINICAL ISOLATES: EFFECTS OF FLUCONAZOLE

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The ability to secrete one or more members of the secretory aspartyl proteinase (Sap) family has been reported as a *Candida* virulence character and a possible correlation of Sap production with antimycotic susceptibility, in these yeasts, may exist. In the present work, production of Sap was assessed in 100 yeast clinical isolates, including 73 strains of *C. albicans*, and the remaining belonging to other species of the genus *Candida*. Isolates were grown on yeast carbon base containing bovine serum albumine (BSA), a Sap-inducing medium, and proteinase production was determined by the size of the halos of hydrolyzed substrate surrounding the colonies. BSA degradation was also confirmed, after 24 and 48 hours of growth, by SDS-PAGE. Five of the *C. albicans* strains showed high levels of Sap activity as well as one strain of *C. tropicalis* and one of *C. parapsilosis*. No activity was detected in *C. krusei* and *C. glabrata*. In parallel, growth on Sap-inducing medium containing 0, 0.25, 0.5 and 1MIC of fluconazole was performed, in selected strains, and cultures sampled daily, for two weeks, to determine extracellular Sap activity by enzymatic degradation of BSA. The results indicated that an exposure to the drug enhanced Sap production in all *C. albicans* isolates tested. To evaluate the effect of the culture medium and the temperature on SAP production, selected strains were grown in different media, with and without BSA and at 25 e 35°C. It was observed that in YEPD no proteolytic activity was detected in *C. albicans* and, regarding temperature, in general, BSA degradation was faster at 35°C. Expression studies of the different genes coding for Saps (*SAP1-9*), were also carried, along the growth curve. Apparently, the genes active during the different growth phases are replaced, suggesting that the different Saps can play distinct roles in the mechanisms of tissue invasion.