**TITLE:** IDENTIFICATION OF CELL-ASSOCIATED AND SECRETED SERINE-TYPE PEPTIDASES IN CLINICAL ISOLATES BELONGING TO THE *Candida haemulonii* COMPLEX

**AUTHORS:** SOUTO, X.M.; RAMOS, L.S.; BRANQUINHA, M.H.; SANTOS, A.L.S.

**INSTITUTION:** UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (LABORATÓRIO DE INVESTIGAÇÃO DE PEPTIDASES, AV. CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, CEP 21941-902, RIO DE JANEIRO-RJ, BRAZIL)

**ABSTRACT:**

In recent years, an increasing number of rare *Candida* species with reduced susceptibility to antifungal agents have been reported, among these, fungi belonging to the *Candida haemulonii* complex (*C. haemulonii*, *C. haemulonii* var. *vulnera* and *C. duobushaemulonii*). Members of this fungal complex, causing superficial and deep infections, are resistant to amphotericin B and various azoles, which hinders the treatment of patients with deep infections and increases the frequency of clinical failures followed by death. Very little is known about the expression of potential virulence markers in this fungal complex. However, it is well-known that *Candida* species express a large array of hydrolytic enzymes, which play roles in fungal-host interaction and some of them present potential therapeutic target. In the present work, we have identified cell-associated and secreted peptidases in nine Brazilian clinical isolates of *C. haemulonii* complex through zymography assay. Peptidases able to hydrolyze gelatin, casein, albumin, hemoglobin and immunoglobulin G were detected in cell-free supernatants (molecular masses ranging from 35 to 85 kDa) and cellular extracts (molecular masses ranging from 35 to 80 kDa) of the three species forming the *C. haemulonii* complex preferentially at physiological pH and 37-42°C. Almost all the peptidase activities were fully inhibited by PMSF, suggesting they refer to serine-type peptidases. However, some minor peptidases were inhibited by both PMSF and 1,10-phenanthroline, suggesting the presence of metal-dependent serine peptidases. Interestingly, the peptidase profiles of *C. haemulonii* and *C. haemulonii* var. *vulnera* isolates were quite similar, contrasting to the peptidases produced by the *C. duobushaemulonii* isolates. Collectively, our results showed for the first time the capability of *C. haemulonii* species complex to produce both cell-associated and secreted serine peptidases able to cleave a broad spectrum of relevant host proteinaceous substrates.

**Keywords:** *Candida haemulonii* complex, serine-type peptidases, peptidase inhibitors

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