TITLE: CHARACTERIZATION OF SECRETED SERINE-TYPE PEPTIDASES FROM CLINICAL ISOLATES BELONGING TO *Candida haemulonii* COMPLEX

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ABSTRACT:

Candida haemulonii complex (*C. haemulonii*, *C. haemulonii* var. *vulnera* and *C. duoushaemulonii*) are emerging fungal pathogens able to cause superficial and deep human infections. Recently, we described the presence of serine peptidases able to degrade several proteinaceous substrates like gelatin, casein, human and bovine albumins, hemoglobin and immunoglobulin G, in both supernatants and cell extracts from nine clinical isolates of *C. haemulonii* complex. These enzymes showed to be completely inhibited by PMSF, presenting higher activity at neutral pH in the range from 37 to 42°C. In order to better characterize the secreted serine-type peptidases of *C. haemulonii* species complex, their activities were assessed in the culture supernatants of three clinical isolates. In this way, firstly, five *p*-nitroanilide-labeled peptide substrates were tested: *N*-Suc-Ala-Ala-Pro-Phe-*p*NA (S1) for chymotrypsin, *N*-Suc-Ala-Ala-Pro-Leu-*p*NA (S2) and *N*-Suc-Ala-Ala-Ala-*p*NA (S3) for elastase, and *N*-Benzoyl-Phe-Val-Arg-*p*NA (S4) and *N*-Benzoyl-DL-4Arg-*p*NA (S5) for trypsin. Serine peptidase activity was detected against all the test substrates, except for S5. Since a higher activity was observed against S1 and S4 substrates, these were then selected to evaluate the effect of pH, temperature, divalent cations and peptidase inhibitors on the enzymatic activity. In general, pHs 7 and 9 were those of greater activity against S1 and S4, respectively. Moreover, serine peptidase activities were highest at 37/45°C for both peptide substrates. Additionally, divalent cations as Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} showed to modulate the serine peptidase activity. With regard to the effect of proteolytic inhibitors, PMSF, AEBSF and 1,10-phenanthroline were able to inhibit the cleavage of both substrates in a typically concentration-dependent manner, while TPCK and benzamidine were effective only against S1 and S4, respectively. The apparent Km values for the serine peptidases detected in the supernatants were also evaluated. In general, the Km for serine peptidases able to degrade S1 ranged from 0.96 to 0.99 mM and for serine peptidases able to degrade S4 ranged from 0.098 to 0.17 mM. Since very little is known about these enzymes in representatives of the *C. haemulonii* complex, these data open new frontiers to future studies in order to better understand its features and performance as a possible virulence factor and assess its viability as therapeutic target.

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

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