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Secreted protease and activity of an endoproteinase for preliminary characterization of clinical *Candida* isolates

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Abstract

Nine clinical *Candida* strains (*Candida dublinienses* (one), *Candida albicans* (six), and *C. tropicalis* (two)) were characterized by production of secreted protease, activity of endoproteinase A and with the HiCandida identification kit. Protease secretion was in the range of 1.3-1.8 cm while PrA activity was between 0.19 and 0.44 µg/min/mg protein. Results are indicative of the possible use of endoproteinase activity and SAP production for characterizing *Candida* strains.

Keywords: Biochemical activity, *Candida*, characterization, endoproteinase, secreted protease.

Introduction

Candida species are the most commonly isolated human opportunistic pathogens, especially in immunosupppressed individuals such as people infected with the Human Immuno-Deficiency Virus (HIV), those using in-dwelling catheters and those with prolonged chemotherapy (Santos *et al*.,2002; Ahmad *et al*.,2004; Kuhn *et al*.,2004).

This study was undertaken with the aim of determining the possible use of secreted protease and activity of an endoproteinase for further characterizing nine clinical *Candida* isolates and to document additional data on *Candida* diversity.

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MATERIALS AND METHODS

***Candida* strains:** Nine *Candida* strains isolated from genital samples (high vaginal swabs and urethra swabs were obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The *Candida* isolates were grown on Yeast Peptone Dextrose, YPD Agar (0.5% yeast extract, 1% peptone, 2% glucose and 2% agar powder, BD France) with added chloramphenicol (50µg/ml) at 37^{o}C for 48 hours.

The strains arbiterally numbered 1-9 were identified as *C. dublinienses* (isolate 1), *C. albicans* (isolates 2, 3, 5, 7, 8 and 9), C. *tropicalis* (isolates 4, 6)). Pure cultures were maintained on YPD slants and stored at 0- 4°C for future use.

***Saccharomyces cerevisae*:** The strain used as control was a diploid standard laboratory strain, a gift from Dr. Anil Ghosh of the Department of Biotechnology, Indian Institute of Chemical Biology, Kolkata. Secreted protease and protease activity were determined according to Santos *et al* (2002) and Lowry *et al* (1951) respectively.

RESULTS

Secreted protease (SAP) was detected in all the nine *Candida* isolates. The diameters of halo (clear zone) surrounding innoculum were in the range of 1.3-1.8 cm (Fig 1). The production of SAP

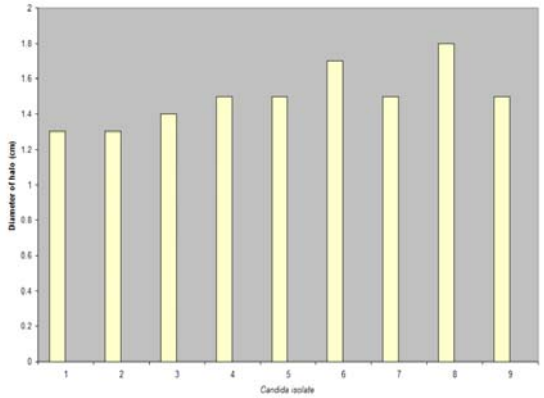

Figure 1: Effect of diameter of halo on nine *Candida* isolates

was not detected in the *S. cerevisae* strain. Activity of protease A (PrA) was observed in all nine *Candida* isolates, ranging from 0.19-0.35 µg/min/mg protein for 15 minutes, and 0.2-0.44 for 30 minutes incubation (Fig 2). Enzyme activity was generally higher at 30°C. There were significant differences in PrA activities of isolates 1 and 2 and other isolates (at 15min incubation) and between those of 3 and 9 and others (at 30min incubation). Enzyme activity was significantly high for isolate 8 (30min incubation). Also, there was significant difference between values obtained for 15 minutes and 30 minutes incubation only for isolates 2, 8 and 9. With the HiCandida kit, six *C. albicans*, two *C. tropicalis* and one *C. dubliniensis* strains were identified. However, further separations were observed using SAP and PrA characterization, producing 2, 3 subgroups (*C.albicans*) and 0, 2 subgroups (*C. tropicalis*), respectively (Table 1).

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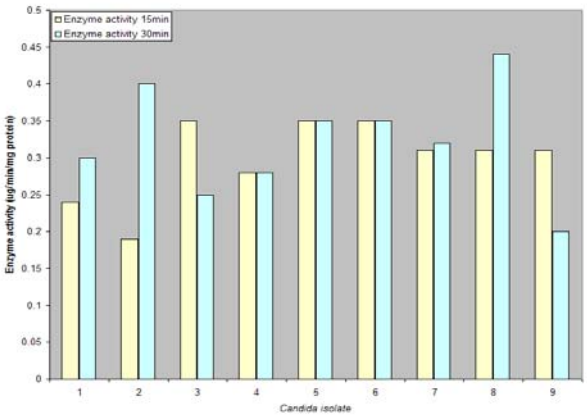

Figure 2: Effect of enzyme activity on nine *Candida* isolates

Discussion and conclusions

Differences in proteolytic activity against albumin by SAP in *Candida* strains, have been used earlier to differentiate them into species and biotypes (Krzeminska – Jaskowiak *et al.,*1994;Vidotto *et al.,*2004). This is the first report of the use of an endoproteinase

Table 1: Speciation of nine *Candida* isolates by HiCandida Kit, SAP and PrA

Candida species	Number of same species identified by			
	Hi-*Candida*	SAP	PrA (15 min)	PrA (30 min)
C.albicans	6	5+1	(1) + (2) + (3)	(2) + (2) + (2)
C.tropicalis	2	2	(1) + (1)	(1) + (1)
C. dubliniensis	1	1	1	1

for characterizing *Candida* spp. There is the possibility of using the combination of SAP and PrA to characterize *Candida* species.

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