CHITINASE FROM *PARACOCCIDIOIDES BRASILIENSIS*: MOLECULAR CLONING, STRUCTURAL, PHYLOGENETIC, EXPRESSION AND ACTIVITY ANALYSIS

Bonfim, S.M.R.C. [a], Cruz, A.H.S. [a], Jesuino, R.S.A. [a], Ulhoa C.J. [b], Molinari-Madlum, E.E.W.I. [c], Soares, C.M.A. [a] and Pereira, M. [a].

[a]Laboratório de Biologia Molecular, Instituto de Ciências Biológicas, Universidade Federal de Goiás, 74001-970, Goiânia-Goiás, Brazil
[b]Laboratório de Enzimologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás, 74001-970, Goiânia-Goiás, Brazil
[c]Laboratório de Imunopatologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, 74001-970, Goiânia-Goiás, Brazil

Corresponding author: Dr. M. Pereira, Laboratório de Biologia Molecular, ICB II, Campus II - Universidade Federal de Goiás, 74001-970, Goiânia-Goiás, Brazil
Tel/fax: 55-62-521-1110

E-mail address: mani@icb.ufg.br

Key words: *Paracoccidioides brasiliensis*, chitinase, enzymatic activity, cellular differentiation.

1. Introduction

Fungi are the causative agents of a wide range of human pathogenesis. Indeed, the last two decades has witnessed a remarkable increase in the incidence of deep-seated, disseminated mycoses (Beck-Cague and Jarvis, 1993). Immune-compromised patients and the development of fungal resistance to conventional treatments have contributed for this scenario (Kontoyiannis and Lewis, 2002; Anaissie, 1992; Corti et al., 2003; Goldani and Sugar, 1995; Hahn et al., 2003). In this way, the search for new drug targets has been necessary. One of the most prevalent fungi in Latin America is *Paracoccidioides brasiliensis*. Over ten million people are estimated to be infected, but only up to 2% develop the disease .

Enzymes involved on the cell wall metabolism have been approached as interesting targets to be explored to the design of specific antifungal agents, since this structure is absent in human .Chitin, a β-1,4-linked polymer of *N*-acetylglucosamine (GlcNAc), is a major component of fungal cell walls. Chitin synthases, chitinases and N-acetyl-β-D-glucosaminidases are hydrolytic enzymes involved in the cell wall metabolism and required for cell growth. In dimorphic fungi, the transition of phases involves changes in the cell wall composition. An increase in chitin levels in parasitic phases is detected in *Candida albicans* and *P. brasiliensis* , hyphae and yeast-like, respectively, defining a cell wall thickness to the fungus inside the host.

2. Materials and methods

2.1. Growth conditions and phase transition
2.2. Generation of a cDNA probe by PCR
2.3. Cloning of the cDNA encoding Pbcts1
2.4. Comparison of sequences and inferred phylogeny
2.5. Southern blot analysis
2.6. Preparation of inocula and infection of mice
2.7. Assay for colony-forming units
2.8. RNA isolation/ Northern blot analysis/. RT-PCR analysis
2.9. Obtaining cellular and secreted fractions of P. brasiliensis cells
2.10. Enzyme assays

3. Results

3.1. Nucleotide and predicted amino acid sequence encoding of Pbcts1
3.2. Characterization of the deduced amino acid sequence
3.3 Phylogenetic analysis
3.4. Genomic organization
3.5. In vitro expression analysis of Pbcts1
3.6. Detection of enzymatic activity in P. brasiliensis

4. Discussion

By searching in the transcriptome of *P. brasiliensis*, we identified an ORF related to a chitinase homologue of this fungus. In this present study, we report the molecular cloning, structural and phylogenetic analysis of the deduced protein. *Pbcts1* encodes a protein with a predicted molecular mass of 45 kDa and calculated p*I* of 6.0. The deduced protein *Pb*CTS1 contains 406 amino acid residues, enclosing in its sequence regions identified as the catalytic residues of the chitinases. The alignment of the deduced amino acid of *Pb*CTS1 with sequences of the chitinases of various fungi reveals a high degree of homology. Here we approached phylogenetic analysis among 79 glycosyl hydrolases family 18 complete chitinase of fungi. Identity and similarity values ranging between 68-31% and 79-46%, respectively, were found when comparing *Pb*CTS1 with all those fungal chitinases. Southern blot analysis performed at high stringency indicated that *Pbcts1* is probable present as a single copy in the *P. brasiliensis* genome. Northern blot analysis showed a single transcript migrating as a mRNA species of 1.9 kb, reinforcing the presence of only one gene in the fungal genome. Although the Northern blot analysis showed the presence of the *Pbcts1* transcript only in the yeast phase, RT-PCR assay shows the presence of transcript in yeast and also in the mycelium phase and transcripts were present in yeast cells obtained from infected mice, RNA processed from yeast cells of *P. brasiliensis* removed from liver of infected mice were used in RT-PCR reaction. The presence of amplification shows that *Pbcts1* is present during infection of host tissues and could be important for the

process.Although *Pb*CTS1 seems not to have N-terminal signal peptide, and has been predicted as a cytoplasmic molecule, it presents an endocytic signal that can also direct traffic within the endossomal and the secretory pathways. The presence of this motif suggests that *Pb*CTS1 could use nonclassical protein secretion pathways *Pb*CTS1 presents an Endosome-Lysosome-Basolateral sorting signal, which sort and internalize signals of proteins to the lysosomal-endosomal-melanosomal compartments. *Pb*CTS1 has Aamy domain, indicating the possibility of presenting alpha amylase activity.Glycosaminoglycan (GAG) attachment site and Laminin G, a domain of binding to heparin, were found in *Pb*CTS1. GAGs are ubiquitous molecules expressed on both in cell surfaces and in extracellular matrix (ECM). The presence of several putative antigenic residues and high hydrophilic index of *Pb*CTS1 suggest its antigenic nature. According to our results, yeast secretes a high quantity of NAGase compared to mycelium. In this way, *P. brasiliensis* is able to use chitin to its maintenance. Small values in the transition from mycelium to yeast suggest housekeeping NAGase activity.

References

Beck-Cague CM and Jarvis WR 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States 1980-1990. *J Infect Dis* 167: 1247-1251.

Kontoyiannis DP and Lewis RE 2002.Antifungal drug resistance of pathogenic fungi. *Lancet* 359: 1135-1144.

Anaissie E 1992.Opportunistic mycoses in the immunocompromised at a Cancer Center and review. *Clin Infect Dis* 14 Suppl.1 S43-S53.

Corti M, Palmieri O, Negroni R, Soto I, Villafane MF 2003. Peripleuritis due to *Paracoccidiodes brasiliensis* in a patient with AIDS. *Medicina* 63: 417.

Goldani LZ, Sugar AM 1995. Paracoccidioidomycosis and AIDS: an overview . *Clin Infect Dis* 21: 1275-1281.

Hahn RC, Morato Conceição YT, Santos NL, Ferreira JF, Hamdan JS 2003. Disseminated paracoccidioidomycosis: correlation between clinical and *in vitro* resistance to ketoconazole and trimetroprim sulphamethoxazole. *Mycoses* 46:342-347.

This work was supported by grants from IFS (International Foundation for Science), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FUNAPE/UFG (Fundação de Apoio a Pesquisa/Universidade Federal de Goiás). Bonfim, S.M.R.C. and Cruz, A.H.S. were recipients of fellowships from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).