PRODUCTION AND CHARACTERIZATION OF A NOVEL ANTIGEN OF *Histoplasma capsulatum* FOR ITS POTENCIAL USE IN DIAGNOSIS AND THERAPEUTICS

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INTRODUCTION

Histoplasmosis is a systemic and endemic mycosis widely distributed in the Americas caused by the dimorphic fungus *Histoplasma capsulatum* [1]. The infection is usually asymptomatic in immunocompetent individuals. However, immunocompromised patients may contract the disseminated form of the disease, which has a bad prognosis and requires rapid diagnosis and treatment. The definitive diagnosis involves the isolation of *H. capsulatum* by culture from clinical specimens, which may take up to 4 weeks. In addition, molecular methods are expensive and have low sensitivity and immunoassays present many false-positive results [2]. Liposomal amphotericin B, which is the treatment of choice for severe cases, is highly expensive. Unfortunately, there is a lack of other therapeutic alternatives for this severe clinical form of histoplasmosis as initial intensive induction treatment. Increased research activity is thus needed to produce and evaluate novel antigen candidates as reagents for diagnosis and to look for new therapeutic targets to develop novel antifungal strategies. In this regard, a 100 kDa protein of *H. capsulatum*, Hcp100, was proposed as a potential new target for histoplasmosis therapy and diagnosis due to its essential role in the fungal adaptation and survival inside the macrophages during infection [3]. Hence, the aim of this work was to express and purify Hcp100 to use it as a possible tool in the diagnosis and follow-up of histoplasmosis and to perform characterization studies in order to engineer new fungal inhibitors with higher efficacy and less toxicity.

MATERIALS AND METHODS

Expression of Hcp100. The gene that encodes for Hcp100 was constructed with a secretory signal and a polyhistidine-tag and expressed in the *Pichia pastoris* X-33 strain. Cell culture supernatants from different induction times were analyzed by SDS-PAGE, Western blot and mass spectrometry.

Purification of Hcp100. Purification of Hcp100 from a 24h cell culture supernatant was carried out using a Ni-NTA affinity chromatography column. Flow-throughs, washes and eluate (500mM Imidazole) were analyzed by 12% SDS-PAGE following *Coomassie-blue* R250 staining and Western blot.

DNA binding assay. DNA binding ability of purified Hcp100 was evaluated by chromatography using DNA-agarose and heparin columns. The input sample, flow-throughs, washes and eluates (1M NaCl) were analyzed by Western blot.

Dot blot assay. An aliquot of 0,5µl of each antigen (purified Hcp100, Histoplasmine, Coccidiodine and Paracoccidiodine) was spotted onto a nitrocellulose membrane. After blocking, membranes were incubated first with 1:100 to 1:10000 dilutions of rabbit polyclonal antibodies against *H. capsulatum, Paracoccidioides brasiliensis* and *Coccidiodes* sp. and then, with goat anti-rabbit IgG horseradish peroxidase conjugate as the secondary antibody. Dots were visualized by using the ECL-Plus detection kit.

Bioinformatics analysis. Similar sequences of Hcp100 retrieved from the National Center of Biotechnology Information using tblastn tool and GBID CAA06786.1 as query were compared to infer orthology ralations using the OrthoMCL and OMA orthology databases. Domains of Hcp100 were predicted using Pfam 31.0 server (EMBL-EBI).

RESULTS

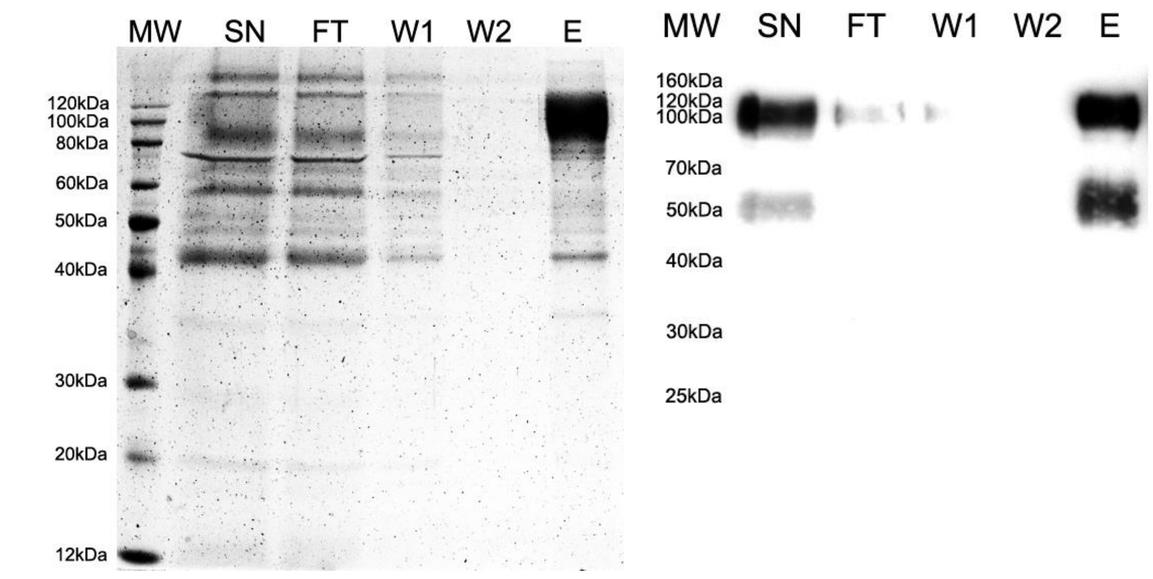

FIGURE 1. **Purification of Hcp100 by Ni-NTA affinity chromatography.** Hcp100 was successfully expressed and purified (~90% purity) from the cell culture supernatant (SN) and its identity was confirmed by Western blot and mass spectrometry. Flow-through (FT), washes (W1 and W2) and eluate (E) were analyzed by 12% SDS-PAGE following *Coomassie-blue* R250 staining (left) and Western blot (right).

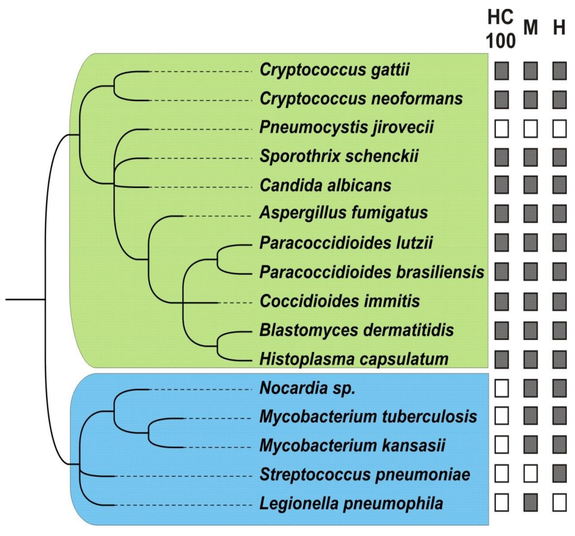

FIGURE 4. **Orthology analysis.** The M and H antigens, currently used in diagnosis of histoplasmosis, are present in several species of fungi and bacteria. On the other hand, Hcp100 has no orthologues in those bacteria that produce similar clinical and radiographic manifestations to those observed in histoplasmosis, indicating that this novel candidate could be more effective for diagnosis.

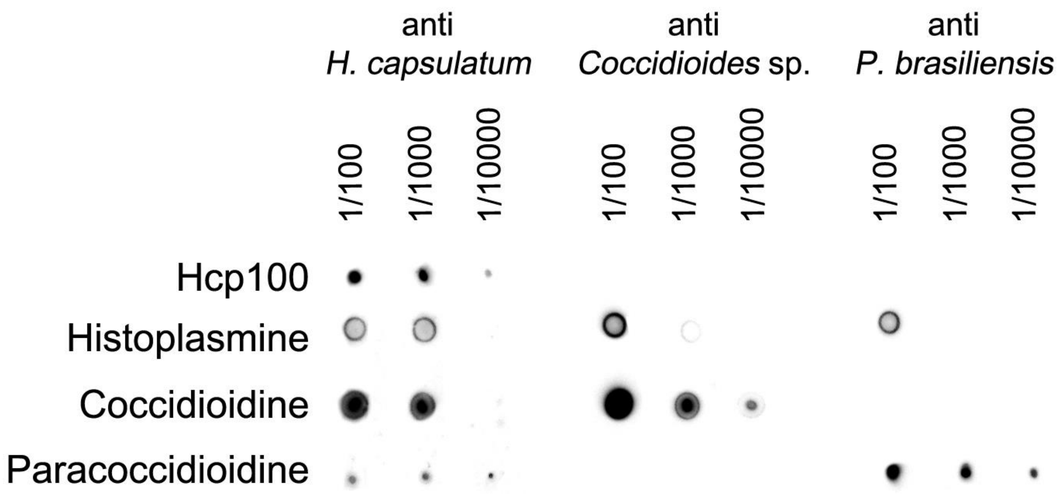

FIGURE 5. **Dotblot.** Hcp100 proved to be immunoreactive against anti-*H. capsulatum* antibodies and no cross-reactions were observed with anti-*P. brasiliensis* and anti-*Coccicíodes* sp. antibodies. On the other hand, cross-reactivity was observed between Histoplasmine and the others fungal antigens, Coccidioidine and Paracoccidioidine, leading Hcp100 as a more feasible marker for diagnosis.

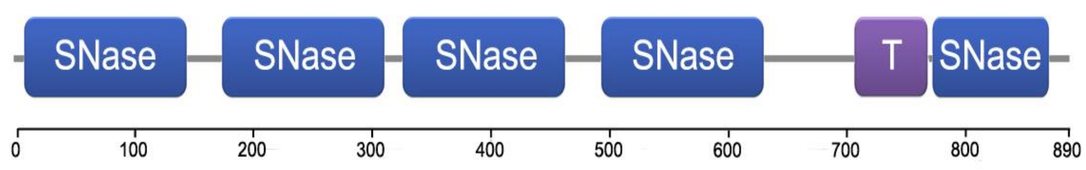

FIGURE 2. ***In silico* characterization of Hc100.** Hc100 is a 890-amino-acid protein with a theoretical molecular weight of 98.6kDa. Four Staphylococcal nuclease(SNase)-like domains of ~150 amino acids each, a shorter SNase like domain of ~100 amino acids and one Tudor domain were predicted using the Pfam 31.0 server (EMBL-EBI). The SN-like domains consists of a subdomain A, which contains an oligonucleotide/oligosaccharide-binding-fold, and a subdomain B containing two alpha helices. However, these SNase-like domains lack the active site residues of nuclease A. The tudor domain is a domain of unknown function that it has been described in several RNA-binding proteins.

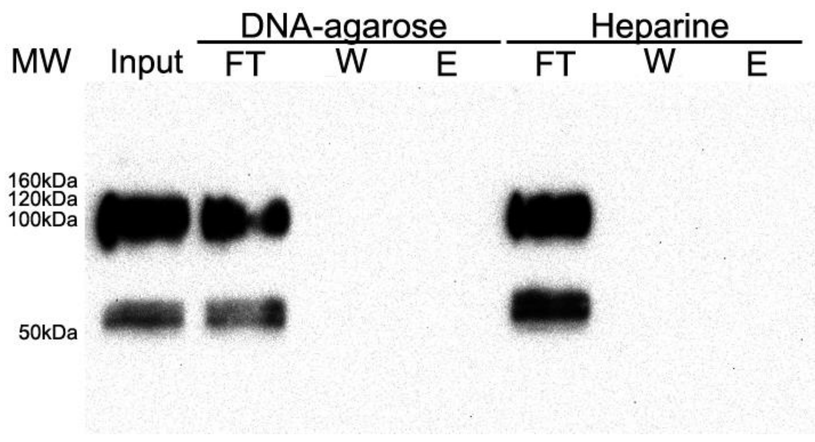

FIGURE 3. **DNA binding assay.** No DNA or heparin binding ability was observed despite the presence of the Oligonucleotide-Oligosaccharide binding fold in this protein, suggesting its possible involvement in protein networks. Input: Sample loaded onto each column, FT: Flow through, W: wash, E: eluate.

CONCLUSIONS

P. pastoris proved to be an excellent system for recombinant Hcp100 expression with potential applications in diagnosis, prognosis and molecular targeted therapy. Further characterization of the different Hcp100 domains are needed so as to elucidate its role in fungal pathogenesis. The Hcp100 protein has proved to be immunoreactive against anti-*H. capsulatum* rabbit polyclonal antibodies. This approach raised the possibility that this protein might be use as a potential diagnostic tool for *H. capsulatum* detection in human samples.

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