The antigens Enolase, Triosephosphate isomerase and Heat shock protein HSS1 of *Mucor circinelloides* are recognized by sera from immunocompromised infected mice

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INTRODUCTION

The genera *Mucor* is the second most common causal agent of mucormycosis, only exceeded by *Rhizopus*. Among them, *Mucor circinelloides* is the most frequently isolated species of the genera. Mucormycosis affects, above all, immunocompromised individuals, and its incidence has increased in the last years. This infection is highly aggressive and spreads quickly, invading the blood vessels, inducing hemorrhages, thrombosis, heart attacks and tissue necrosis. In addition, its diagnosis is usually carried out at a late stage of infection, and the treatment strategies are not clear.

OBJECTIVE

The aim of this work was the **identification of the most immunogenic antigens of *M. circinelloides***. An innovative approach using immunosuppressed mice was employed to detect them so that only the most immunoreactive were selected.

RESULTS

1. Identification of the antigens of the secretome

The proteomics study of the secretome of *M. circinelloides* by 2-dimensional electrophoresis (2-DE) showed that secreted proteins were localized throughout the whole isoelectric point (p*I)* range used and with Molecular weights (Mr) smaller than 130 kDa. Specifically, the majority of the proteins contained in the secretome presented a p*I* between 4.5-7, and a Mr between 25-70 kDa (Fig. 1a). From this extract, using sera of immunocompromised infected mice, the immunoreactivity was analyzed and the seven most immunoreactive antigens were identified by mass spectrometry. Three of these spots corresponded to Enolase and four to Triosephosphate isomerase, as shown in the Table 1. They are isoforms of the Enolase and Triosephosphate isomerase, respectively.

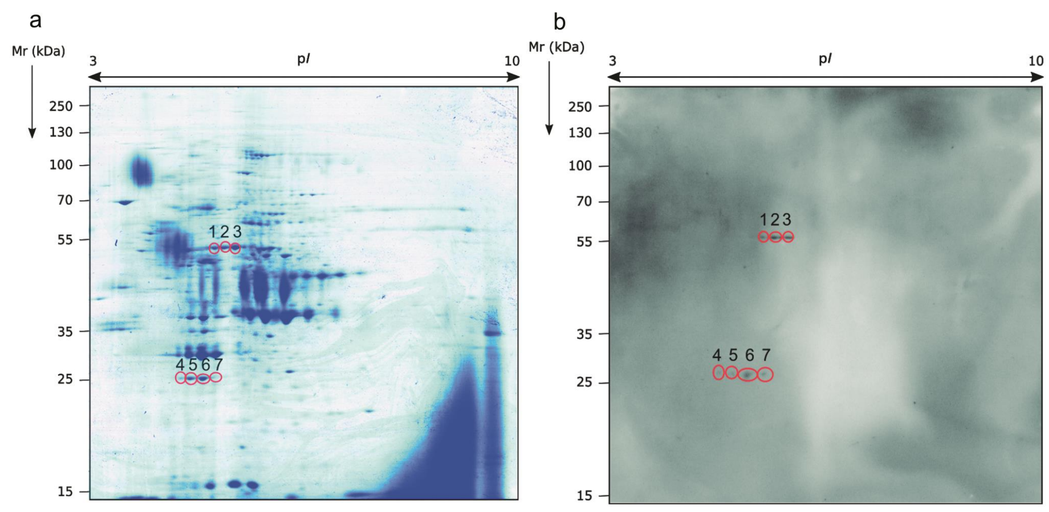

Figure 1. Analysis of 2-DE of the secretome (a, b) of *Mucor circinelloides*. a, stained with Coomassie Brilliant Blue. b, WB obtained using the sera of immunocompromised infected mice.

Triosephosphate isomerase was not identified in *M. circinelloides*, but in the fungus of the same genus *Mucor ambiguus*. When comparing this protein sequence with the genome of *M. circinelloides*, an identity value of 99.48% was found, indicating the high degree of similarity given between both sequences of Triosephosphate isomerase of *M. ambiguus* and *M. circinelloides.* This enzyme has also been identified in the fungi *Candida albicans* and *Paracoccidioides brasiliensis*, and is considered an important antigen, capable of binding the laminin and fibronectin of the extracellular matrix. Moreover, it can be found in the cell wall, but also in the secreted extracellular vesicles.

Table 1. Analysis of the antigens identified in the secretome of *Mucor circinelloides*.

Spot number	NCBI number	Name of the protein	Microorganism	Matches	Cover (%)	Score	Theorethical pI/Mr(kDa)	Experimental pI/Mr(kDa)
1	EPB85979.1	Enolase	*Mucor circinelloides*	10	34	855	5,56/47,17	5,44/54,14
2	EPB85979.1	Enolase	*Mucor circinelloides*	14	36	1093	5,56/47,17	5,65/54,66
3	EPB85979.1	Enolase	*Mucor circinelloides*	4	12	317	5,56/47,17	5,76/54,66
4	GAN00908.1	Triosephosphate isomerase	*Mucor ambiguus*	6	27	319	5,26/26,95	5,23/26,28
5	GAN00908.1	Triosephosphate isomerase	*Mucor ambiguus*	10	32	537	5,26/26,95	5,00/26,57
6	GAN00908.1	Triosephosphate isomerase	*Mucor ambiguus*	14	48	807	5,26/26,95	5,24/26,79
7	GAN00908.1	Triosephosphate isomerase	*Mucor ambiguus*	11	40	663	5,26/26,95	5,50/26,79

2. Identification of the antigens of the total extract

The study of the fungal cell proteome by 2-DE of the total extract showed that the proteins were also localized on the whole range of p*I* and Mr used. However, the observed protein distribution pattern observed was very different from that of the secretome and almost all proteins were present in the ranges of p*I* of 5-9 and Mr of 40-70 kDa (Fig. 2a). Finally, the immunome detected by two-dimensional Western Blot (WB) showed a very few number of immunoreactive proteins recognized by sera from immunosuppressed mice and, therefore, only the two most immunoreactive spots were selected for their further identification (Fig. 2a and 2b). These proteins were identified by mass spectrometry as Enolase and Heat shock protein HSS1 (Table 2).

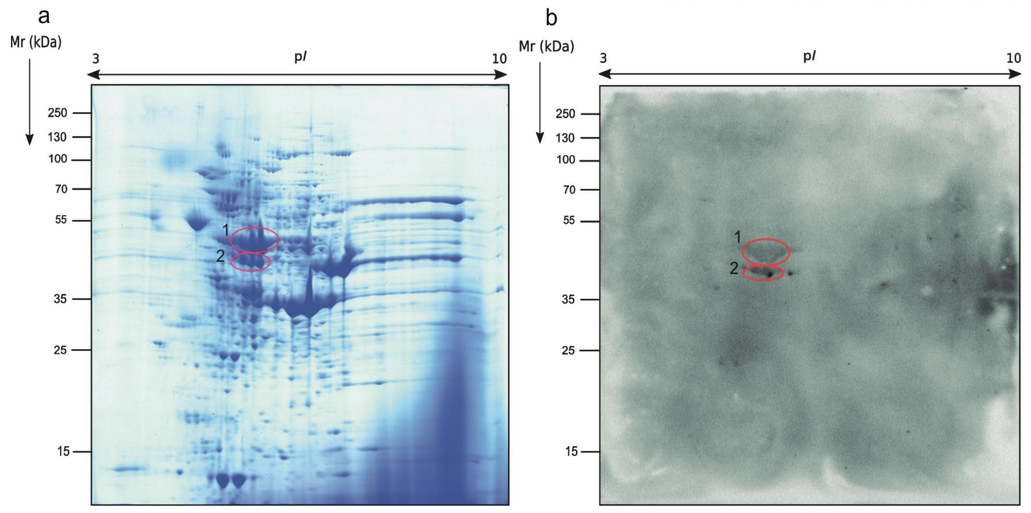

Figure 2. Analysis of 2-DE of the total extract (a, b) of *Mucor circinelloides*. a, stained with Coomassie Brilliant Blue. b, WB obtained using the sera of immunocompromised infected mice.

HSS1 belongs to the same heat shock protein family as the Hsp70, which in fungal species is overexpressed during infection to prevent the denaturalization of proteins and has been described as immunoreactive against serum IgGs and mucosal IgAs. This protein could be considered an orthologue of the Hsp70 proteins, SSA1 and SSA2, found in the ascomycetous fungi *C. albicans*, and the Hsp70 of *Rhizopus arrhizus*, as it presents an identity value of 78.58%, 76.43% and 66.39%, respectively.

Table 2. Analysis of the antigens identified in the total extract of *Mucor circinelloides*.

Spot number	NCBI number	Name of the protein	Microorganism	Matches	Cover (%)	Score	Theorethical pI/Mr(kDa)	Experimental pI/Mr(kDa)
1	EPB85979.1	Enolase	*Mucor circinelloides*	45	46	1346	5,56/47,17	5,79/50,88
2	EPB91082.1	Heat-shock protein HSS1	*Mucor circinelloides*	23	33	1530	5,07/70,92	5,79/46,23

Enolase was identified in both analyzed extracts

Enolase deserves a special mention as it has been identified in both analyzed protein extracts. This metabolic enzyme was also previously identified by our research group as an antigen recognized by salivary IgAs in *Lomentospora prolificans,* showing cross-reactivity with *Aspergillus fumigatus,* and by serum IgGs in *C. albicans*. Enolase was also associated with the cell wall in *C. albicans* and in *L. prolificans.* Its sequence was compared with the sequence in *C. albicans, R. arrhizus* and *Homo sapiens*, and similarities of 69%, 81.88% and 71% were found, respectively.

CONCLUSIONS

- The **most immunoreactive antigens of *M. circinelloides*** identified **using sera of immunocompromised infected mice** were **HSS1**, **Enolase**, and **Triosephosphate isomerase**.
- They **can be useful for the development of a vaccine, antifungal treatments and/or for diagnosis**, allowing the rapid detection and treatment and lowering the unacceptable mortality rates.

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