Triosephosphate isomerase of *Mucor circinelloides* is recognized as a main secreted antigen by sera from infected immunosuppressed mice

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INTRODUCTION

Mucormycosis, infections caused by fungi of the order *Mucorales*, are a group of emerging infectious diseases characterized by rapid progression and high aggressiveness. The genera *Mucor* is one of the most common etiological agents, being *Mucor circinelloides* the most frequently isolated species. Moreover, their virulence mechanisms are still unknown and there is no effective diagnostic method for this disease apart from classical methods.

OBJECTIVE

The aim of this study was to identify the secreted antigens of *M. circinelloides* recognized specifically by immunosuppressed infected mice sera.

RESULTS & METHODS

1. Protein obtention

Mucor circinelloides was grown into potato dextrose broth at 37°C with 120 rpm agitation for 24 hours. After that, the fungal material was separated from the medium by filtration and incubated at 37°C with 120 rpm agitation in PBS 2% glucose for 20 hours.

Then, the supernatant was filtered, sterilized and dialyzed, and finally, proteins were precipitated by TCA/acetone method and resuspended in buffer for two-dimensional electrophoresis (2-DE).

Fig. 1. Diagram of the workflow used for the obtention of the secretome proteins from *M. circinelloides*.

2. Protein separation and identification

Protein separation was carried out by 2-DE and antigen detection was performed by Western Blot against a pool of sera obtained from 5 mice infected intravenously with 1×10^5 conidia of *M. circinelloides* NRRL 3631 and 5 control sera from non-infected mice- All of the mice were immunosuppressed by giving them 100 mg/kg cyclophosphamide intraperitoneally at -4 days and, then, every three days until the day 20 in which the mice were sacrificed and the sera was obtained.

Eight immunoreactive proteins were specifically detected by sera from infected immunosuppressed mice. Four of them were detected at isoelectric point (pI) of 5,4-5,7 and a molecular weight (Mr) of 50 kDa, which were identified as enolase by LC-MS/MS. The other four spots were the most immunoreactive and were identified as triosephosphate isomerase at 4,8 -5,4 pI and Mr 25 kDa.

Fig. 2. Two-dimensional proteome (A) and immunome (B) analysis of the secreted proteins.

Table 1. Identified antigens of *M. circinelloides* in secretome.

Spot	Identified protein
1, 2, 3, 4	Triosephosphate isomerase
5, 6, 7, 8	enolase

CONCLUSIONS

- Triosephosphate isomerase was identified, together with enolase, as a major secreted antigen of *M. circinelloides*.
- The study in depth of the immunoreactivity of this antigen should be accomplished to analyze its potential as target for diagnosis.

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