Lomentospora prolificans cyclophilin and enolase are recognized by immunocompetent human IgA as the most prevalent conidial antigens and cross-react with *Aspergillus fumigatus*

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INTRODUCTION

The fungus *Lomentospora prolificans* is a emergent pathogen[1] which mainly affects immunocompromised patients, causing disseminated infections, with a mortality rate close to 90%[2]. This mortality is mainly associated with the fungus intrinsic resistance to commonly used antifungals and with the clinical conditions of the patients.

It is worth to highlight that the immune system of immunocompetent individuals seems to be able to avoid these severe infections. In that case, after the inhalation of conidia, mucosa secrete a great deal of proteins into the lumen, being IgA the most important, which prevents the adherence of microorganisms and the colonization of the airway epithelium.

OBJECTIVE

The objective of this work was to identify the most **prevalent conidial antigens** of *L. prolificans* recognized by human salivary IgA, to study their **localization** and their **presence on phylogenetically related fungi**, *Scedosporium apiospermum* and *Scedosporium aurantiacum*. Finally, **cross-reactivity study** was performed between antigens of *L. prolificans* and the most common filamentous pathogen *Aspergillus fumigatus*.

RESULTS

Identification of the most prevalent antigens

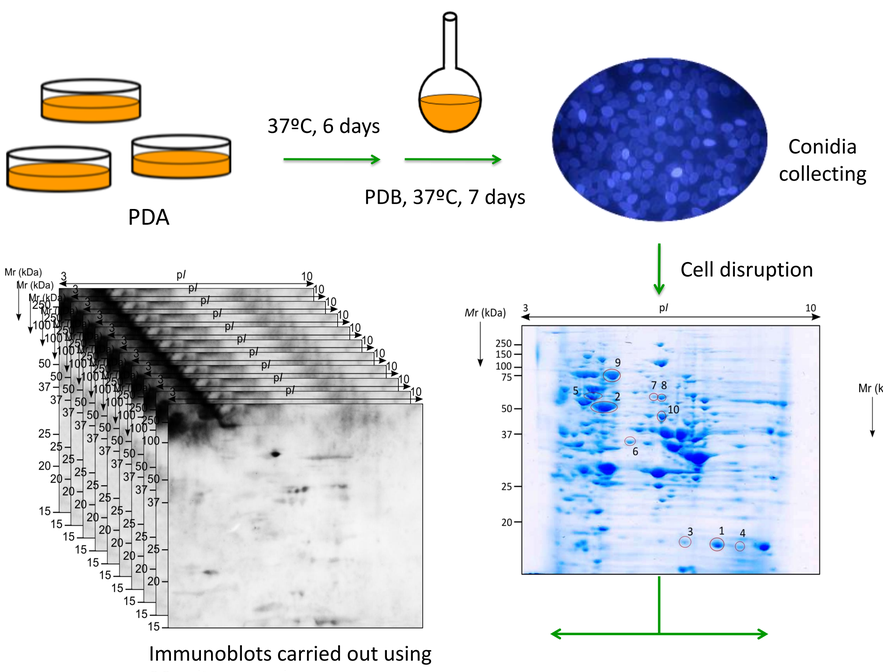

Fig. 1. Diagram of the workflow used for the identification of the most prevalent antigens of *Lomentospora prolificans* recognized by human salivary IgA.

The detection of the immunogenic proteins of *L. prolificans* recognized by human salivary IgA was performed using two-dimensional electrophoresis (2-DE) and Western Blot (WB)[3]. The immunomes obtained using each of the 20 saliva collected from immunocompetent individuals were compared with the one obtained using a pool of all the saliva to study the prevalence of each antigen (Fig. 1).

Then, 10 antigens that reacted with more than 50% of the saliva samples were identified by LC-MS/MS (Table 1). Among these antigens, **cyclophilin** and **enolase** showed to be the most prevalents, being recognized by 85% and 80% of the saliva, respectively. Moreover, all the saliva samples used exhibited reactivity against at least one of the two proteins, and thus, antibodies against those enzymes would give 100% of coverage to *L. prolificans*.

Table. 1. Identification and seroprevalence of the antigens from *Lomentospora prolificans* that have been detected by more than 50% of saliva samples.

Spot no.	Prevalencia (%)	Proteína identificada	*Spot* no.	Prevalencia (%)	Proteína identificada
1	**85**	**Putative mitochondrial cyclophilin 1**	**6**	60	Malate dehydrogenase
2	**80**	**Enolase**	**7**	60	Dihydrolipoamide dehydrogenase
3	60	Cyclophilin type peptidyl-prolyl cis-trans isomerase/CLD	**8**	55	Hypothetical protein MYCTH_2309210 (dihydrolipoyl dehydrogenase)
4	60	40S ribosomal protein S18	**9**	55	Heat shock 70 kDa protein
5	60	Hypothetical protein (tubulin)	**10**	55	Putative branched-chain-amino-acid aminotransferase TOXF

Identification of enolase and cyclophilins as cell wall antigens

Cell wall proteins were extracted by incubating conidia for 10 min at 100ºC in extraction buffer with SDS. Then, proteins were separated by 2-DE, and WB was performed against the human saliva pool (Fig. 2).

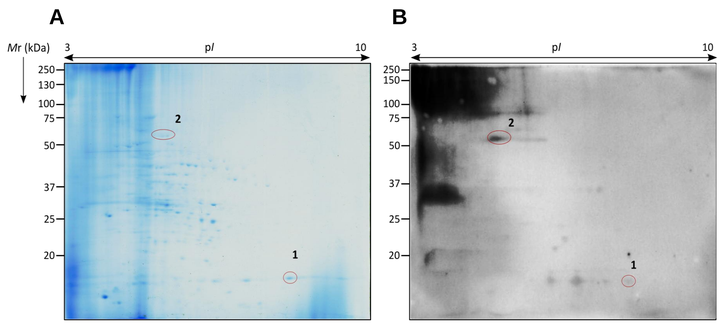

Fig. 2. Two-dimensional proteome **(A)** and immunome **(B)** analysis of conidial cell wall proteins.

Enolase (spot 1) and **cyclophilins** (spot 2) were detected and identified in the cell wall proteome by LC-MS/MS (Fig. 2), where cyclophilins appeared to be one of the most abundant proteins. Furthermore, both enolase and cyclophilins showed to be surface antigens, being strongly recognized in the cell wall immunome by human salivary IgA from immunocompetent donors.

Comparative study of *L. prolificans*, *S. aurantiacum* and *S. apiospermum* immunomes

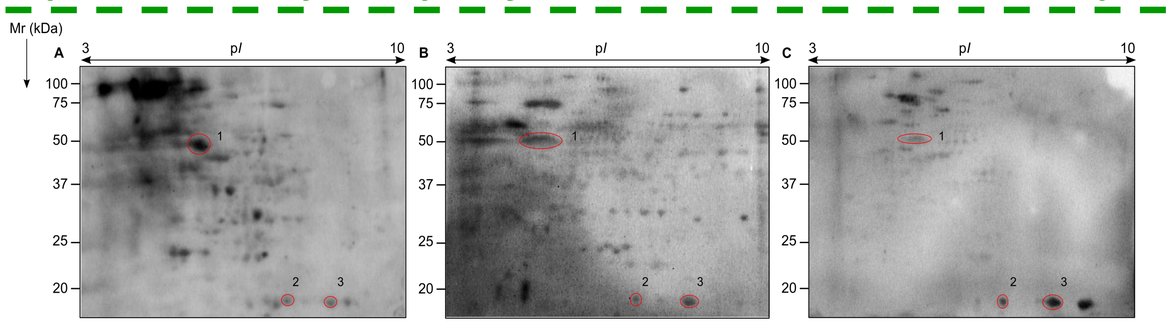

Fig. 3. Salivary **i**mmunome of *Lomentospora prolificans* (**A**), *Scedosporium apiospermum* (**B**) and *Scedosporium aurantiacum* (**C**) obtained against human saliva pool.

To detect the prevalent antigens identified in this study on other species of the *Scedosporium* complex, comparative studies were carried out with conidial immunomes of *L. prolificans, S. apiospermum* and *S. aurantiacum* (Fig. 3), which were selected because of their phylogenetic proximity and clinical relevance.

Despite the differences observed, the most important antigens of *L. prolificans*, **enolase** (spot 1) and **cyclophilins** (spot 2 and 3) were also detected and identified on *S. apiospermum* and *S. aurantiacum* immunomes with high intensity.

Cross-reactivity between *L. prolificans* and *A. fumigatus*

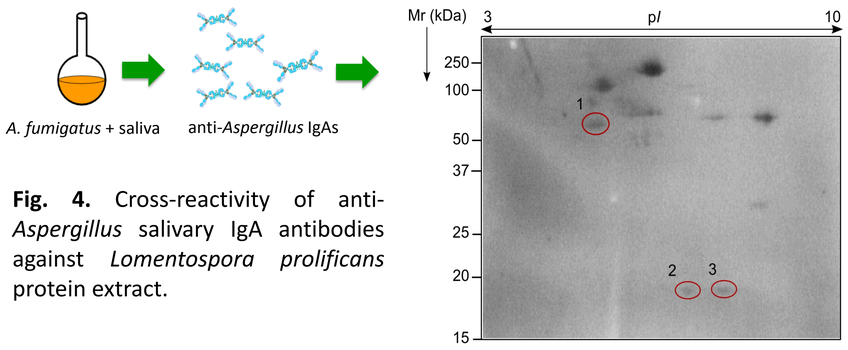

Fig. 4. Cross-reactivity of anti-*Aspergillus* salivary IgA antibodies against *Lomentospora prolificans* protein extract.

Cross-reactivity was studied using the most common spore-producing fungus, *A. fumigatus*. For this, conidia from *A. fumigatus* were incubated in a saliva pool for 2 h at 37ºC and, then, anti-*Aspergillus* antibodies were recovered and blotted with the *L. prolificans* extract (Fig. 4).

The *L. prolificans* **enolase** (spot 1) and **cyclophilins** (spot 2 and 3) were detected using 2-DE and WB by the anti-*Aspergillus* antibodies.

CONCLUSION

- ✓ The **enolase** and **cyclophilins** are the most prevalent antigens of *L. prolificans* recognized by immunocompetent saliva and they are located on the cell surface.
- ✓ They have also been detected and identified in *S. aurantiacum* and *S. apiospermum*, and purified anti-*Aspergillus* antibodies have specifically recognized them.
- ✓ According to these findings, the enzymes enolase and cyclophilin seem to be interesting to be evaluated as therapeutic targets and immunogens for developing vaccines, which might be also protective to other fungal pathogens because the cross-reactivity showed.

References

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