Production of *Scedosporium boydii* CatA1 and SodC recombinant proteins: another step towards a standardized test for serodiagnosis of *Scedosporium* infections

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Introduction

Scedosporium species are responsible for a wide variety of infections ranging from localized infections to disseminated infections in severely immunocompromised patients, particularly patients with cystic fibrosis (CF) undergoing lung or heart-lung transplantation. Serodiagnosis of these infections may be useful to confirm the biological diagnosis in deep-seated chronic infection, but also in CF patients with a *Scedosporium*-positive sputum culture to differentiate between an airway colonization and a respiratory infection. Nevertheless, it is limited to specialized laboratories using crude antigenic extracts because of the lack of commercially available serodiagnosis kits. Therefore, it may be hampered by variations from one batch of the antigenic extract to another, and cross-reactions with other pathogenic fungi including the aspergilli. To overcome these limitations, we investigated, using recombinant proteins, the antibody response in several groups of patients with or without an *Aspergillus fumigatus* or a *Scedosporium* infection, and evaluated their value as serodiagnostic tools to discriminate *Aspergillus* and *Scedosporium* infections and to differentiate between an airway colonization by *Scedosporium* and a respiratory infection.

Production of *Scedosporium* recombinant proteins

Recombinant proteins mimicking *Scedosporium boydii* catalase A1 (rCatA1) or cytosolic superoxide dismutase (rSodC) or *S. apiospermum* alkaline phosphate (rAlp1) or dipeptidylpeptidase V (rDPPV) and tagged with histidine (His) or with glutathione-S-transferase (GST) were produced in *Pichia pastoris* or *Escherichia coli*. All purified recombinant proteins showed, by SDS-PAGE, a molecular mass consistent with the expected mass (100, 45, 60, 100 and 26 kDa for rGST-SbCatA1, rGST-SbSODC, rGST-SaAlp1, rGSTSaDDPV and rGST, respectively), except rSbCatA1-(His)₆ which exhibited a molecular mass of 100 kDa instead of the 80-kDa expected mass.

Fig. 1: Schematic representation of the recombinant proteins produced in *Escherichia coli* or *Pichia pastoris*.

Reactivity of human sera against *Scedosporium* recombinant proteins

Sera from group A correspond to CF patients without any filamentous fungus recovered from sputum samples during the 6 months preceding or following blood sampling; sera from group B correspond to CF patients colonized by *A. fumigatus* and with a positive antibody response exclusively against *A. fumigatus*; sera from group C correspond to CF patients colonized only by *Scedosporium* species, but without antibodies directed against *Scedosporium* species; sera from group D correspond to CF patients with an exclusive recovery of *Scedosporium* species from respiratory secretions and with a serological response exclusively against *Scedosporium* species antigenic extract. Only rCatA1 and rSodC allowed the detection of *Scedosporium* infection, and the differentiation with an *Aspergillus* infection. Combination of the results showed a sensitivity of 88.2% and a specificity of 97%.

Table 1: Positivity of patient sera against rGST-SbCatA1 and rGST-SbSODC.

	Seropositivity using		
	rGST-SbCatA1	rGST-SbSODC	Both recombinant proteins
Sensitivity	88.2%	88.2%	82.4%
Specificity	97%	97.6%	100%
Positive predictive value	75%	79%	100%
Negative predictive value	99%	99%	98.2%

Conclusion

These recombinant proteins allow the differentiation between *Scedosporium* and *Aspergillus* infections, as well as between airway colonization and infection due to *Scedosporium* species, which may be particularly helpful for clinicians to guide them in their therapeutic decision.

Fig. 2: SDS-PAGE analysis of the recombinant proteins produced in *Escherichia coli* or *Pichia pastoris*.