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Distinct galactofuranose antigens in the cell wall and culture supernatants as a means to differentiate *Fusarium* from *Aspergillus* species

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ABSTRACT

Detection of carbohydrate antigens is an important means for diagnosis of invasive fungal infections. For diagnosis of systemic Aspergillus infections, galactomannan is commonly used, the core antigenic structure of which consists of chains of several galactofuranose moieties. In this study, we provide evidence that Fusarium produces at least two distinct galactofuranose antigens: Smaller amounts of galactomannan and larger quantities of a novel antigen recognized by the monoclonal antibody AB135-8. In *A. fumigatus*, only minor amounts of the AB135-8 antigen are found in supernatants and in the apical regions of hyphae. A galactofuranose-deficient *A. fumigatus* mutant lacks the AB135-8 antigen, which strongly suggests that galactofuranose is an essential constituent of this antigen. Using a combination of AB135-8 and a galactomannan-specific antibody, we were able to unambiguously differentiate *A. fumigatus* and Fusarium hyphae in immunohistology. Moreover, since Fusarium releases the AB135-8 antigen, it appears to be a promising target antigen for a serological detection of Fusarium infections.

1. Introduction

Diagnostics of invasive fungal infections currently relies on culture, histology and/or detection of circulating cell wall carbohydrates (De Pauw et al., 2008). Two major antigens of filamentous fungi are of particular importance and can be detected by serological assays: β-D-glucan and galactomannan. β-D-glucan is a major cell wall component of many fungal pathogens and a positive β-D-glucan assay is therefore not indicative for a specific pathogen. Galactomannan is commonly used for diagnosis of invasive aspergillosis, since it is only produced by certain fungal pathogens, e.g. Aspergillus and Penicillium (Swanink et al., 1997).

Fusarium is an important fungal pathogen in particular in plants, but also in vetebrates. In humans, the most pathogenic species belong to the *F. solani*, *F. oxysporum* or *Gibberella fujikuroi* complex, and from the latter, *F. verticilloides* and *F. proliferatum* are of particular clinical importance (Nucci and Anaissie, 2007). For an efficient therapy, it is important to accurately differentiate Fusarium and Aspergillus, since both differ in their sensitivity to commonly used anti-fungals (Alastruey-Izquierdo et al., 2008). However, an unambiguous identification is hampered by a similar hyphal morphology (Morrison, 2002) and the fact that the β-D-glucan assay is positive for both pathogens. The galactomannan assay (PLATELIA Aspergillus EIA) and its key component, the monoclonal antibody EB-A2, were originally reported to discriminate between Aspergillus and Fusarium (Swanink et al., 1997; Stynen et al., 1992; Cummings et al., 2007). In recent times, Fusarium infections with a positive galactomannan test have been reported (Mikulska et al., 2012; Tortorano et al., 2012; Horn et al., 2014; Kebabcı et al., 2014; Nucci et al., 2014) and in one study, galactomannan was additionally found in Fusarium culture supernatants (Tortorano et al., 2012). This implies that the PLATELIA Aspergillus EIA is not always able to discriminate between Aspergillus and Fusarium infections.

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Fungal galactomannan contains galactofuranose (Gal*f*) as a characteristic constituent and the monoclonal antibody EB-A2 was shown to recognize chains of at least four β-1,5-linked Gal*f* moieties present in Aspergillus cell wall polysaccharides, glycoproteins and certain glycolipids (Stynen et al., 1992; Tefsen et al., 2012). Generation of Gal*f*–containing structures requires synthesis and subsequent transport of UDP-bound Gal*f* into the Golgi apparatus and finally incorporation of Gal*f* into distinct glycostructures by a panel of UDP-galactofuranosyl-transferases (Tefsen et al., 2012). In *A. fumigatus*, the first step is mediated by the UDP-Gal*f* mutase GlfA (Schmalhorst et al., 2008), while GlfB is the UDP-Gal*f* transporter (Engel et al., 2009). Several types of Gal*f* linkages exist in *A. fumigatus* (Bahia et al., 1997), but from the corresponding UDP-galactofuranosyl-transferases only one has been identified so far (Komachi et al., 2013).

In this study, we were able to detect galactomannan in supernatants of several Fusarium species, and using the novel monoclonal antibody AB135-8 we have identified a distinct Gal*f*-dependent antigen of unknown structure. This new antigen is strongly expressed by all Fusarium species tested, whereas *A. fumigatus* hyphae produce it in only minor amounts. In combination with a galactomannan-specific antibody, AB135-8 turned out to be a valuable tool for the immunohistological diagnosis of mould infections.

2. Materials and methods

2.1. Strains

The following strains were used in this study: *F. oxysporum* (DSM 62316), *F. solani* (CBS 131394), *F. dimerum* (NRZ Sch63), *F. proliferatum* (NRZ 2311), *F. andiyazi* (CBS 134430), *F. graminearum* (strain 8/1) (Miedaner et al., 2000), *A. fumigatus* ATCC46645 and its GFP-expressing derivative (Meier et al., 2003), *A. fumigatus* D141, Δ*glfA* mutant and the corresponding complemented strain (Schmalhorst et al., 2008), *A. niger* (DSM 737), *A. nidulans* (FGSC A26), *A. terreus* (SBUG844), *Mucor circinelloides* (CBS 277.49), *Lichtheimia corymbifera* (ATCC 46771), *Rhizopus oryzae* (DSM 1185), *Scedosporium minutisporum* (strain 01-0564), *S. boydii* (strain 01-0715), *S. aurantiacum* (strain 07-0433), *S. apiospermum* (strain 08-0118.01), *Microsporum canis* (VMT 1046) and *Trichophyton interdigitale* (VMT 761).

2.2. Fungal cultures and isolation of conidia

For isolation of conidia, *A. fumigatus* was grown on AMM (Aspergillus Minimal Medium) agar in tissue culture flasks (Sarstedt, Nümbrecht, Germany). Conidia were harvested using glass beads and sterile water. For *Fusarium*, 100 ml of Sabouraud broth were inoculated with 1.5×10^4 conidia. After 3 d incubation at 30 °C and 140 rpm, the culture was harvested by centrifugation. *Mucorales* were grown on supplementary minimal medium (SUP) agar (10 g glucose, 1 g NH_4Cl, 0.25 g $MgSO_4 \times 7H_2O$, 4 g KH_2PO_4, 0.9 g K_2HPO_4, 5 g yeast extract and 15 g agar ad 1L). All other fungi were grown on Sabouraud agar. Conidia were harvested using buffer containing 0.9% NaCl and 0.001% Tween 20. Conidia suspensions were passed through two layers of Miracloth (Merck, Darmstadt, Germany) to remove larger fungal fragments and the spore concentration was subsequently determined using a Neubauer chamber.

2.3. Sequence analysis

Protein sequences from *A. fumigatus* Af293 were obtained from the Aspergillus Genome Database (http://www.aspgd.org/). Genome searches for *Fusarium* and *Mucorales* were performed using the Fusarium Comparative Database at the Broad Institute (http://www.broadinstitute.org/annotation/genome/fusarium_group/MultiHome.html) and the Saccharomyces Genome Database (http://www.yeastgenome.org/cgi-bin/blast-fungal.pl), respectively. Alignments were generated using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2).

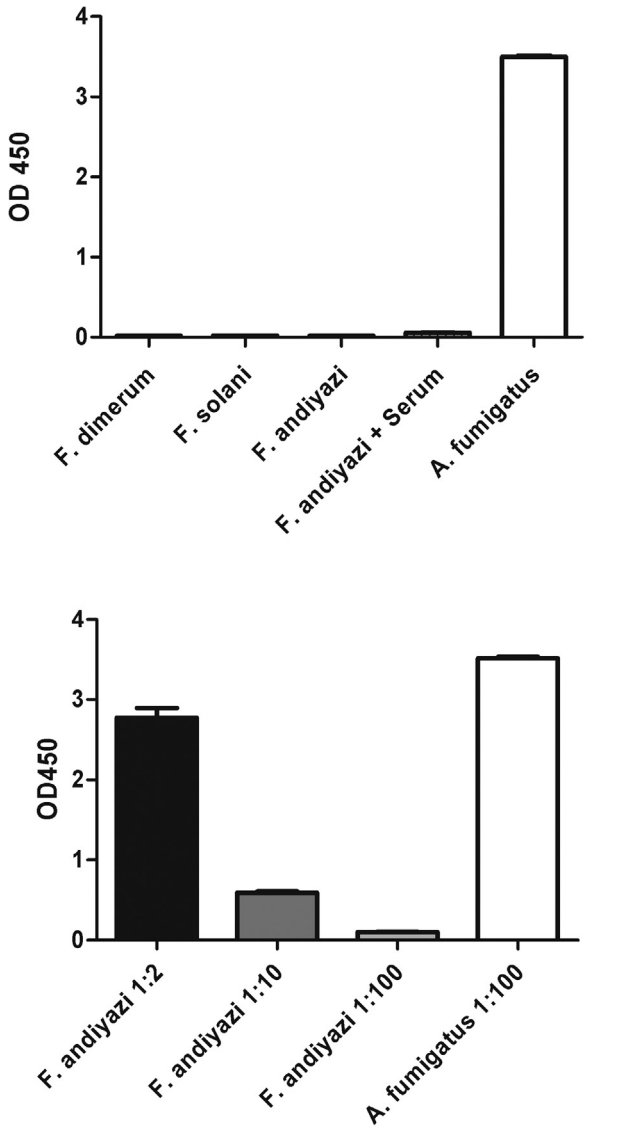

Fig. 1. Detection of galactomannan in culture supernatants of *Fusarium* ssp. and *A. fumigatus*. Cell-free supernatants were harvested after growth in Sabouraud medium (where indicated supplemented with 3% human serum). Samples were diluted in coating buffer (Panel A: 1:100; Panel B: as indicated). The galactomannan content was measured using the PLATELIA Aspergillus EIA.

2.4. Monoclonal antibodies

AB135-8 was identified and cloned from a pool of hybridoma cells obtained after immunization of Balb/c mice with killed *A. fumigatus* germ tubes. The immunoglobulin subclass was determined using the Pierce Rapid Antibody Isotyping Kits (ThermoScientific, Dreieich, Germany). Hybridoma cells were grown in Opti-MEM medium (Life Technologies) supplemented with 5% fetal calf serum. IgM antibodies were purified from culture supernatants using the Pierce IgM Purification Kit (ThermoScientific) according to the instructions of the vendor. The galactomannan-specific monoclonal antibody EB-A2 was initially described by Stynen et al. (1992).