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Mannoproteins from yeast and hyphal form of *Candida albicans* considerably differ in mannan and protein content

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ABSTRACT

Significant differences in carbohydrate composition of mannoproteins obtained from yeast and hyphal cell walls of *Candida albicans* (serotypes A and B) were found. Yeast mannoproteins from both serotypes consisted up to 46% of mannan while the same parts from hyphal cells contained only about 14% of mannan. Another difference was in protein content, 47–53% for yeasts, 3–4.5% for hyphae, respectively. Moreover, HPLC profiles of yeast mannoproteins were more complex compared to those of hyphal form. Subsequently, mannans were prepared from yeast and hyphal mannoproteins using cetavlon fractionation. Mannans from both yeast serotypes contained higher amounts of mannose (91.4% serotype A; 92.8% serotype B) than mannans from hyphae (66.4% serotype A; 76.3% serotype B). Unlike mannans from serotype B, mannans from serotype A contained β-(1→2)-linked mannopyranosyl units in acid-stable moiety. Further, hyphal mannans were less branched than yeast mannans. The shift from yeast to hyphal form probably led to simplification of mannan structure.

1. Introduction

Candida albicans is the most common fungal commensal microorganism in healthy individuals and at the same time the major fungal pathogen in immunocompromised patients. Normally, it colonizes the human skin and mucosal surfaces without apparent deleterious effects. However, when the host defense is weakened *C. albicans* causes mucosal and systemic infections.[1,2]

C. albicans is a polymorphic fungus capable to undergo a morphology switching from the unicellular yeast form to the filamentous forms, pseudohyphae and true hyphae.[3] It was found that the human innate immune system can discriminate between the yeast and hyphal forms of *C. albicans*.[4] It seems that the morphological shift is a critical step in the pathogenicity of this opportunistic fungus. The architecture of *C. albicans* cell wall on formation of mannoprotein complex,[5–8] cell wall proteins,[9] and mannan structure[10–14] has been closely studied over the last decades. It is necessary to consider that these results are related to the yeast form of the pathogen. Thus, the outer layer of the yeast form of *C. albicans* cell wall consists of N- and O-linked mannosylated proteins and the inner layer contains chitin skeletal polysaccharide to which a matrix of β-(1→3)-glucan branched with β-(1→6)-glucan is attached. Glucans are the major components of fungal cell wall and the key fungal pathogen-associated molecular patterns.[4] It has been suggested that *C. albicans* masks underlying β-(1→3)-glucan with a dense layer of mannan and/or mannoprotein and protects the pathogen against host immune system.[15] Despite the relevance of *C. albicans* hyphae to pathogenicity, there are very few reports on the composition of *C. albicans* hyphal cell wall. Using single-molecule atomic force microscopy, Beaussart et al.[16] showed that the yeast-to-hypha transition leads to a major increase in the distribution, adhesion, unfolding, and extension of Als adhesins and their associated mannans on the cell surface. Lowman et al.[17] found that *C. albicans* hyphae possess glucan structures that are unique to the hyphae and are not detected in yeast.

The aim here was to compare the carbohydrate composition and protein content of mannoproteins obtained by the same procedure from the yeast and hyphal cells of *C. albicans* serotype A and serotype B. Moreover we compared the structural similarity and difference of mannans prepared from the individual mannoproteins.

2. Material and methods

2.1. Microorganisms and cultivation conditions

C. albicans serotype A (CCY 29-3-100) and *C. albicans* serotype B (CCY 29-3-103) yeast strains were from the Culture Collection of

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Yeasts (CCY, Institute of Chemistry of Slovak Academy of Sciences, Bratislava, Slovakia). Yeast forms of both *C. albicans* strains were obtained after cultivation on YPD medium containing 0.5% yeast autolysate, 1% peptone, and 2% D-glucose for 48 h at 28 °C using rotatory shaker (100 rpm). Hyphal forms of both *C. albicans* strains were obtained after cultivation on synthetic medium (pH 7.5) containing 1.5% potato starch, 0.5% saccharose, 0.25% K_2HPO_4, 0.05% $NaNO_3$, 0.1% L-methionine, 0.05% L-phenylalanine, 0.05% (w/v) N-acetyl-D-glucosamine, and 0.5 mg/mL of biotin. The static cultivation was at 37 °C for 48 h. Then the yeasts and hyphae were harvested by centrifugation and washed twice with distilled water.

2.2. Preparation of mannoproteins

Wet biomasses of *C. albicans* strains were extracted by autoclaving for 25 min at 120 °C using 0.2 M NaCl. The autoclaving was repeated twice more. Supernatants from individual strains were combined and dialyzed against distilled water. Mannoproteins were precipitated with 0.1% CH_3COONa in ethanol (1:4), dissolved in distilled water, dialyzed, freeze-dried and characterized for carbohydrate and protein content. The samples were labeled as YMP-A from yeast form and HMP-A from hyphal form of *C. albicans* serotype A, and YMP-B from yeast form and HMP-B from hyphal form of *C. albicans* serotype B (Fig. 1, Table 1).

Table 1
Monosaccharide and protein composition of yeast and hyphal mannoproteins from *C. albicans* serotype A and serotype B

	Yeast mannoproteins		Hyphal mannoproteins	
	C. albicans ser. A (YMP-A) (%)	*C. albicans* ser. B (YMP-B) (%)	*C. albicans* ser. A (HMP-A) (%)	*C. albicans* ser. B (HMP-B) (%)
Mannose	45.6	41.9	13.4	13.9
Glucose	4.3	3.1	82.3	80.4
Ribose	2.2	1.5	0.8	0.9
Arabinose	0.2	0.1	0.1	0.1
[a]Protein	46.9	53.1	3.1	4.5

(YMP-A)—yeast mannoprotein serotype A; (YMP-B)—yeast mannoprotein serotype B.
(HMP-A)—hyphal mannoprotein serotype A; (HMP-B)—hyphal mannoprotein serotype B.
[a] Protein content (%N×6.25) was determined from the amount of nitrogen as determined by elemental analysis.

2.3. Preparation of mannans

Mannans were prepared by fractionation of YMP-A, YMP-B, HMP-A and HMP-B with hexadecyltrimethylammonium bromide (cetavlon).[18,19] From each type of extract two fractions were

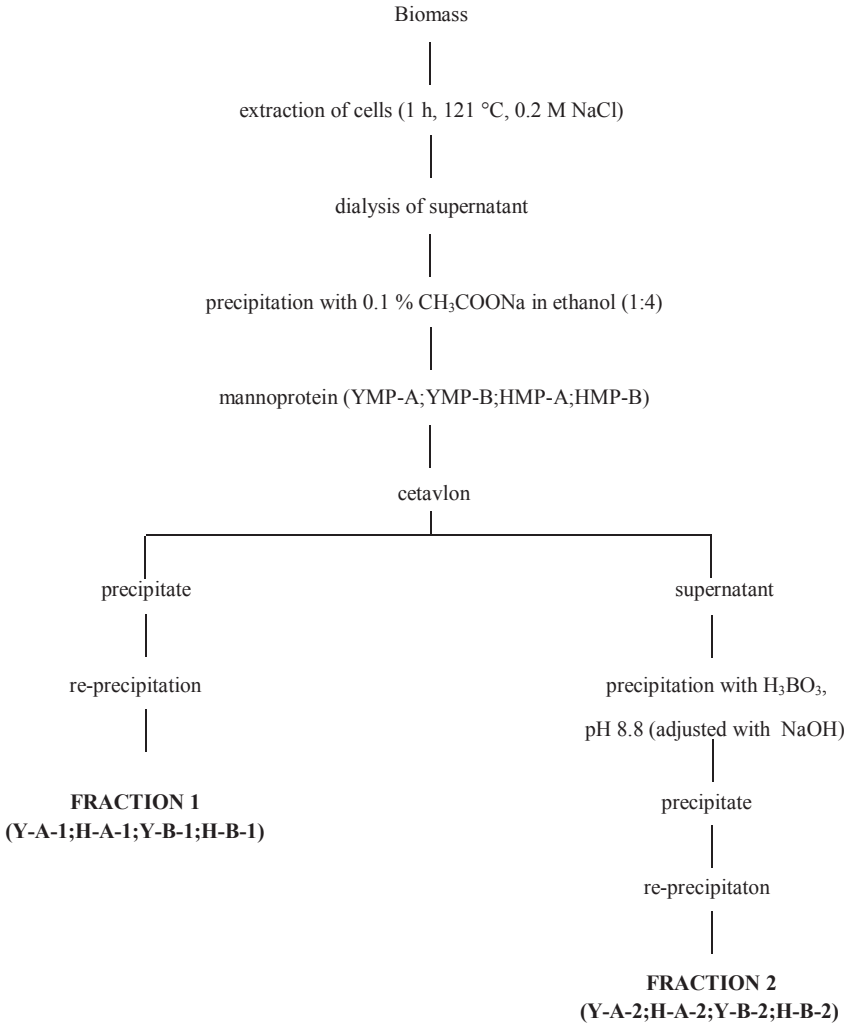

Fig. 1. Preparation of mannoprotein and mannans from yeast and hyphal form of *C. albicans* serotype A and serotype B using hexadecyltrimethylammonium bromide (cetavlon).