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Candida auris mannan from cultivation on Shibata medium

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Abstract

Candida auris, an emerging multidrug-resistant yeast pathogen, is responsible for numerous high-mortality healthcare-associated outbreaks worldwide. In addition, *C. auris* rapid spread is linked to the successful colonization of human skin, leading to bloodstream infections. *C. auris* mannan is different from other pathogenic *Candida* species in its abundance of *β*-1,2-Man-linkages. The mannans are potent immunogens, and as such, the structure of the mannans and their epitopes have significant relevance to pathogenesis. Here we extracted the mannan from *C. auris* and characterized it using NMR, FTIR techniques to understand and analyze the structural differences at 37 °C. We have compared the mannan at 28 °C and 37 °C by FTIR, where we have found no significant differences. However, from TOCSY-NMR we can assume that: 1, both mannans have the same structural parts, 2, the mannans have altered relative abundance of branches.

Keywords: *Candida auris*; *Mannan*; *Cultivation*; *Shibata medium*; *NMR*; *FTIR*

Introduction and Objectives

Candida auris, a critical emerging fungal pathogen, was firstly described in 2009 and has since spread across six continents as a causative microorganism of hospital-acquired infections [1]. *C. auris* is one of the most challenging emerging human pathogens discovered in the last decade for various reasons. It is highly resistant to many commonly used antifungal drugs and has spread rapidly worldwide in just a few years [2]. *C. auris* complicates routine microbiological identification and is difficult to eradicate in healthcare settings. This is due to its ability to colonize the skin, spread via the patient-to-patient route or contaminated fomites, and high survival on plastic surfaces and in the hospital environment [3]. *C. auris* infections are generally associated with the same risk factors as other types of *Candida* infections, such as prolonged hospitalization, exposure to broad-spectrum antibiotics or antifungal agents [4]. Surprisingly, despite chlorhexidine washing, *C. auris* colonization of human skin is challenging to eliminate with its resistance to ethanol and other disinfectants and finish with the necessity to use very strong bleach (10 % NaOCl) and the remarkable persistence of the infection on inanimate surfaces in hospitals and nursing homes necessitates using bleach as the disinfectant [5]. Even with washing, alcohol, and disinfectants containing iodine, *C. auris* adheres firmly to the skin, eliminating numerous other organisms that generally comprise a healthy microbiome. It is unknown which proteins or tissues *C. auris* interacts with once inside

the body. The need for further study to understand the mechanism of its infection, with an emphasis on the role of its cell wall in its pathogenicity.

Nearly every element of the biology and pathogenicity of pathogenic yeasts depends on the cell wall, which is the outer layer of the organism and contains over 60 % carbohydrates. *N*- and *O*-linked mannans with *α*-1,2-, *α*-1,3-, *α*-1,6-, and *β*-1,2-linked mannopyranose units and phospho-linked mannopyranose units make up the components of cell wall mannoproteins [6]. These mannoproteins are potent immunogens that, during candidiasis, activate and regulate the host innate immune response via the mannose receptor, DC-SIGN (dendritic cell-specific intracellular adhesion molecule-3 (ICAM-3) grabbing nonintegrin), and Dectin-2 [7]. Glycan structures vary among different *Candida* species. *Candida* mannans and mannoproteins are potent immunogens and proper vaccines in animal models of candidiasis. A further diagnostic marker for invasive *Candida* infections is fungus mannan antigenemia. The *Candida* cell wall is divided into two layers: an outer layer of highly mannosylated proteins (mannoproteins) and an inner layer made up primarily of *β*-1,3-, *β*-1,6-glucans, and chitin [8]. However, the cell wall, mannoproteins, mannans, and glycome of *C. auris* still need to be better understood [9].

Our goal of this work is to analyze the changes in the structure of *C. auris* mannan from cultivation at 37 ºC in Shibata medium. The structure of mannan in *C. albicans* yeast and hyphal form (the most common morphological state at 37 °C, similar to human body) is different, we investigate whether the similar structural change of mannan is fundamental for *C. auris*.

Materials and methods

Strains and media

Candida auris (CBS 15279, Belgium) strain was taken directly from the frozen stock and passaged on YPD (1 % yeast extract, 2 % peptone, 2 % dextrose, 2 % agar) plate media at and 37 °C. Cells were passaged at shibata medium (2 % glucose, 0.25 % (w/v); K_2HPO_4, 0.05 % (w/v) $NaNO_3$, 0.1 % (w/v) L-methionine, 0.05 % (w/v) L-phenylalanine, 0.05 % (w/v) *N*-acetyl-D-glucosamine, 0.5 ng/mL D-biotin; pH 7.5) for 48 h. And the first cultivation was done in 250 mL × 2 (500 mL) of the medium while the second cultivation was done by passaging the cells in 100 mL shibata for 24 h at 37 °C and then cultivated in 4 × 300 mL of Shibata medium. For the mannan extraction, cells were inoculated in the liquid Shibata medium at 37 ºC at 120 RPM.

Mannan extraction

Mannan was isolated from cells using a modified extraction procedure [10]. Thus, the *C. auris* biomass was suspended in 0.2 M NaCl and autoclaved 3 × for 1 h. Each time the supernatant was collected. Then the mannoprotein was precipitated using 3 vol. of EtOH. Pellet was suspended in H_2O and then dialyzed with 2 % KOH. Then the supernatant was saved and subjected to Fehling's reagent for precipitation. The resulting precipitate was then dialyzed again with 3 M HCl and methanol + acetic acid. After the sample was lyophilized, we obtained product designed as mannan-37.

FTIR analysis

Samples were prepared after isolation of mannan and the lyophilized sample was directly measured on NICOLET Magna 6700 (Thermo Fisher Scientific, USA) spectrometer with DTGS detector and an experimental accessory – Smart Orbit, and OMNIC 8.0 software was used. Infrared spectral analyses were carried out in the mid-infrared region (from 4000 cm^{-1} to 400 cm^{-1}), and spectral data obtained were presented as absorbance values. The number of scans was set to 128. Diamond Smart Orbit ATR accessory was used for measurements in the solid state.

NMR Spectroscopy

After being dissolved in 500 μL of D_2O (99.97 % D), the samples were lyophilized to substitute the exchangeable protons with deuterium and then transferred to NMR microtubes. NMR spectra results were recorded in ^1H NMR and ^1H–^1H 2D TOCSY spectra at 45 °C on a Bruker AVANCE III HD X 600 MHz spectrometer (Bruker TopSpin, Rheinstetten, Germany) equipped with a triple inverse TCI H-C/N-D-05-Z liquid He-cooled cryoprobe and processed using MeStreNova 14 software. The ^1H signal of acetone (2.217 ppm) was used as a reference for chemical shifts. After processing the mannan NMR spectra, structural alterations were identified by comparing the assigned peak intensities. The ranges are height adjusted to each spectrum's highest peaks for each pair of comparisons.

Result and discussion

Growth of cells

On YPD media with 2 % agar, *C. auris* produces smooth and white cream-colored colonies. *C. auris* cells appear to have a small morphology under a microscope without forming pseudohyphae (see Fig. 1). On the other hand, it may exhibit a variety of morphological phenotypes under different culture conditions, including round-to-ovoid, elongated, and pseudohyphal-like forms [11]. Overnight growth in 500 mL and 1,2 L of medium was required

to extract enough mannan for analysis. Whereas, yield of mannan recover 0.4 mg from the first cultivation and 19.2 mg from the second cultivation (mannan-37).

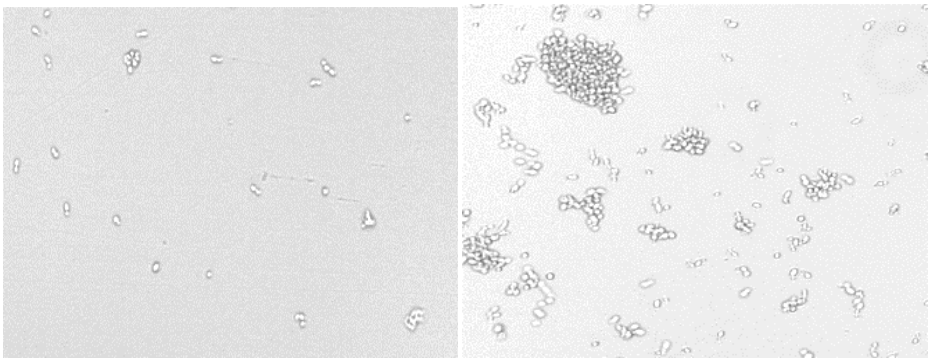

Fig. 1. Growth of *C. auris* in shibata medium at 37 °C for 48 h. under microscope. Cells growth of 1st cultivation (a), cells growth of 2nd cultivation (b).

Fourier-transform infrared spectroscopy

FTIR spectroscopy is a valuable instrument for monitoring structural changes in biopolymers. We used this method to compare mannans from *C. auris* cultivated at 28 ºC and 37 ºC in the 1600–1700 cm^{-1} range and the anomeric region is from 840–860 cm^{-1}. Fig. 2. shows the FTIR spectra of the obtained mannans. The main difference is presence of the ester band at 1737 cm^{-1} in mannan from cultivation on Shibata medium at 37 °C. Amide I band of the mannan assignment with influential bands (stretching vibration of C=O at 1650 cm^{-1}). The maximum band at 1015 cm^{-1} is in the specific region for C—O, C—C, and ring vibration stretching vibration (866–1188 cm^{-1}) carbohydrates assignments. The bands at 850 cm^{-1} in the anomeric region are unique for α-1,2-mannans. No other or major changes were found.

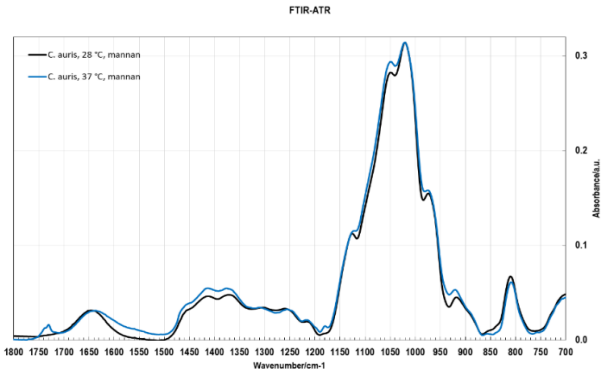

Fig. 2. FTIR spectra of the mannans of *C. auris* cultivated at 28 °C and 37 °C.

NMR spectroscopy (Nuclear Magnetic Resonance)

The sequential assignment of the H-1 and H-2 signals was performed using TOCSY NMR experiment. Although spectra showed different side-chain lengths containing α-1,2-mannose, α-1,3-mannose, and β-1,2-mannoses. Cross peak at 5.35 ppm in ^{1}H spectrum of the mannan-37 indicated traces of phosphate-linked side chain. If we compare our results to the standard cultivation, Manβ1→2Manβ1→2Manα1→2 epitops are appr. 7 % of all mannoses present, but in case of mannan-37, they are 12 %. Manβ1→2Manα1→2 epitopes decreased from 4 % to 2 % for standard mannan and mannan-37, respectively. Also, under standard cultivation mannose units are presented more often as branches as main chain. Unlike in *C. albicans* species, where under such conditions hyphae are formed, and resulting mannans are lack of β1→2 epitops, here are these epitopes preserved.

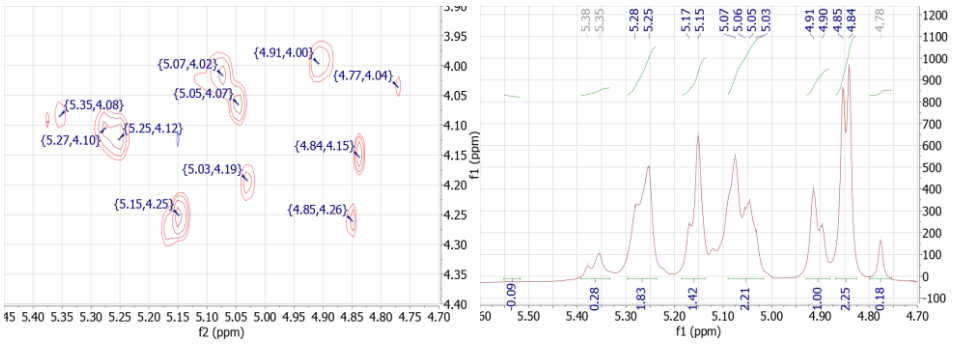

Fig. 3. TOCSY NMR experiment on mannan-37, from *C. auris* cultivated at 37 °C in Shibata medium.

Conclusion

In presented research we concluded that it is difficult to yield the satisfactory amount of mannan within cultivation at 37 °C. Data interpretation after FTIR, NMR analysis did not show crucial differences of mannans structures, only some alternation in relative epitopes abundance. This fact is intriguing because other species, e.g., *C. albicans*, transform the mannan structure substantially. In the future, we extend our findings by further independent experiments and provide a prerequisite for gaining unknown insights into the carbohydrate of *C. auris* factors of virulence.

Acknowledgments

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