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# Mannan structural complexity is decreased when *Candida albicans* is cultivated in blood or serum at physiological temperature

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ABSTRACT

The *Candida albicans* cell wall provides an architecture that allows for the organism to survive environmental stress as well as interaction with host tissues. Previous work has focused on growing *C. albicans* on media such as Sabouraud or YPD at 30 °C. Because *C. albicans* normally colonizes a host, we hypothesized that cultivation on blood or serum at 37 °C would result in structural changes in cell wall mannan. *C. albicans* SC5314 was inoculated onto YPD, 5% blood, or 5% serum agar media three successive times at 30 °C and 37 °C, then cultivated overnight at 30 °C in YPD. The mannan was extracted and characterized using 1D and 2D $^{1}$H NMR techniques. At 30 °C cells grown in blood and serum contain less acid-stable terminal β-(1→2)-linked D-mannose and α-(1→2)-linked D-mannose-containing side chains, while the acid-labile side chains of mannan grown in blood and serum contain fewer β-Man-(1→2)-α-Man-(1→ side chains. The decrement in acid-stable mannan side chains is greater at 37 °C than at 30 °C. Cells grown on blood at 37 °C show fewer →6)-α-Man-(1→ structural motifs in the acid-stable polymer backbone. The data indicate that *C. albicans*, grown on media containing host-derived components, produces less complex mannan. This is accentuated when the cells are cultured at 37 °C. This study demonstrates that the *C. albicans* cell wall is a dynamic and adaptive organelle, which alters its structural phenotype in response to growth in host-derived media at physiological temperature.



## 1. Introduction

The opportunistic pathogen *Candida albicans* is one of the most commonly isolated organisms as a hospital-acquired infection in critical care wards.[1,2] *C. albicans* is a commensal organism readily isolated from human skin and has several virulence traits, including the production of hydrolytic enzymes, and adhesins as well as the ability to undergo a morphological shift from yeast to hyphal morphologies,[3–5] which allow it to be pathogenic in immunocompromised individuals. In addition, one of the major contributors to *C. albicans* virulence is its cell wall, which serves a major function in protecting the organism from environmental stress as well as acting as a support structure for attachment of adhesion molecules and other virulence-associated proteins. In addition, the fungal cell wall serves as the interface with the environment in which the cell grows, such as a human host. Recent studies have demonstrated that the fungal cell wall is recognized by a number of host specific receptors that allow for an appropriate immune response.[6,7]

The *C. albicans* cell wall is composed of chitin (a polymer of *N*-acetylglucosamine) that is attached to a matrix of β-D-(1→3)-glucan. The β-D-(1→3)-glucan polymer has a number of β-D-(1→6)-glucan branch points, which serve as linkage sites via a glycophosphatidylinositol remnant and internal repeat (PIR) moieties for N- and O-linked mannosylated protein attachment.[8] It has been suggested that *C. albicans* masks the underlying cell wall β-D-(1→3)-glucan with a dense layer of mannan and/or mannoprotein.[9] As β-D-glucan is the primary fungal pathogen associated molecular pattern (PAMP),[10–12] this 'masking' or covering the glucan with mannan/mannoprotein is thought to reduce recognition of the yeast by anti-fungal innate immune mechanisms, such as via recognition by Dectin-1.[6] The *Candida* cell wall has been extensively studied, but most of these investigations have focused on defining cell wall structure following cultivation in medium such as YPD or Sabouraud agar.[13–21] While these data have advanced our knowledge, they have not addressed the question of what structural changes occur in the *Candida* cell wall in response to cultivation in complex biological media, such as blood or serum. Indeed,

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little is known about the changes that occur in the cell wall composition and architecture as a result of cultivating fungi on different media and under different environmental conditions, such as growth at physiologic temperature (37 °C).

In this study, we compared and contrasted the structure of mannan/mannoprotein in the cell wall of *C. albicans* strain SC5314 grown in blood, serum, or YPD. In addition, we also compared and contrasted the effect of cultivation temperature on mannan/mannoprotein structure. To the best of our knowledge, this is the first in depth investigation of how growth conditions, that is, temperature and medium, impact the structural phenotype of mannan/mannoprotein in the *C. albicans* cell wall. When considered as a whole, our results indicate that the *C. albicans* cell wall mannan structure is complex, dynamic, and highly adaptable. We speculate that changing the structural phenotype of the cell wall may confer a survival advantage to the organism.

## 2. Results and discussion

Over more than a decade, several groups[18,22–29] have made great strides in understanding the structure of mannan isolated from fungal cell walls of several *Candida* species. By using elegant 2D NMR experiments (up to 600 MHz) these investigators examined the structural details of mannan side chains after carefully degrading the mannan and isolating the side chain fragments. Vinogradov and co-workers[30] used 750 MHz NMR to extend these structural studies on intact mannan isolated from *Saccharomyces cerevisiae* with and without degradation followed by isolation of the fragmented side chains. Maes and co-workers[31] used solid-state magic-angle-spinning NMR at 800 MHz to examine mannan structures in intact cell walls of live *C. albicans* cells. Using solid-state NMR, Maes and co-workers observed the anomeric proton resonance for α-Man-1→$PO_4$, that is, a single mannosyl repeat unit attached to the phosphodiester linkage. However, Maes and co-workers[31] and others[18,22–30,32] did not report identification of this linkage in previous solution-state NMR studies.

### 2.1. Growth on different culture media at different temperatures alters mannan structure

In this study we utilized 1D and 2D COSY and NOESY NMR experiments at 950 MHz (Figs. S1–S3) as well as previously published chemical shift assignments characteristic of individual mannosyl motifs in specific side chain fragments based on 2D NMR studies of isolated side chain fragments[22–28,30–32] to assign proton NMR resonances to structural motifs of non-degraded, intact mannans. Specifically we correlated the unique chemical shifts of the anomeric proton, H-1, and its neighboring proton, H-2, in specific mannosyl repeat units of isolated mannan side chain fragments to the chemical shifts of mannosyl repeat units in similar chemical

**Table 1**
Changes in mannan structural motifs as a function of growth in either blood or serum and growth temperature during the three passages at either 30 °C or 37 °C and final growth at either 30 °C or 37 °C relative to growth in YPD under the same conditions based upon the chemical shifts (in ppm) for the anomeric protons of the mannosyl repeat units shown in Bold.

| Label | $^1$H Chemical shifts (ppm) | | Structural motifs | 30 °C/30 °C[a] | | 37 °C/30 °C | | 37 °C/37 °C | |
|---|---|---|---|---|---|---|---|---|---|
| | H-1 | H-2 | | Blood[b] | Serum[b] | Blood[b] | Serum[b] | Blood[b] | Serum[b] |
| **a** | 5.572 | 4.219 | β-Man-(1→2)-α-**Man**-(1→$PO_4$ | ⇓[c] | ⇓ | = | ⇑ | ⇑ | ⇑ |
| **b** | 5.560 | 4.207 | β-Man-(1→2)-[β-Man-(1→2)]$_n$-β-Man-(1→2)-α-**Man**-(1→$PO_4$ | = | = | = | ⟺ | ⇑ | ⇑ |
| **c** | 5.471 | 4.033 | α-**Man**-(1→$PO_4$ | = | ⇑ | ⇑ | ⟺ | ⇑ | ⟺ |
| **d** | 5.385 | 4.109 | α-Man-(1→2)-α-**Man**-(1→3)-α-Man-(1→ | ⇓ | = | ⇓ | ⇓ | = | ⇓ |
| **e** | 5.303 | 4.120 | α-Man-(1→3)-α-Man-(1→2)-α-**Man**-(1→3)[α-Man-(1→6)]-α-Man-(1→2) | ⇓ | ⇓ | ⇓ | ⇓ | ⇓ | ⟺ |
| **f** | 5.297 | 4.135 | α-Man-(1→3)[α-Man-(1→6)]-α-Man-(1→2)-α-**Man**-(1→3)[α-Man-(1→6)]-α-Man-(1→2) | ⇓ | ⇓ | ⇓ | ⇓ | ⇓ | ⟺ |
| **g** | 5.286 | 4.132 | α-Man-(1→2)-α-**Man**-(1→2) | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **h** | 5.266 | 4.110 | α-Man-(1→2)-α-**Man**-(1→2) | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **i** | 5.179 | 4.290 | β-Man-(1→2)-α-**Man**-(1→ | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **j** | 5.162 | 4.271 | β-Man-(1→2)-α-**Man**-(1→2) | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **k** | 5.120 | 4.048 | α-**Man**-(1→6)[d] | ⇓ | ⇓ | ⇓ | ⇓ | —[e] | —[e] |
| **l** | 5.107 | —[e] | α-**Man**-(1→6)[d] | ⇓ | ⇓ | ⇓ | ⇓ | —[e] | —[e] |
| **m** | 5.088 | 4.089 | α-**Man**-(1→6)[d] | ⇓ | ⇓ | ⇓ | ⇓ | —[e] | —[e] |
| **n** | 5.067 | 4.083 | α-Man-(1→2)<br>→6)[α-Man-(1→2)-α-**Man**-(1→2)]-α-**Man**-(1→6) | = | = | = | = | = | = |
| **o** | 5.045 | 4.222 | α-Man-(1→3)-α-**Man**-(1→2)-α-Man-(1→ | = | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **p** | 4.940 | 4.171 | β-Man-(1→2)-[β-**Man**-(1→2)]$_n$-α-Man-(1→[f]<br>β-Man-(1→2)-β-**Man**-(1→2)-β-Man-(1→2)-α-Man-(1→$PO_4$ | = | = | = | = | ⇑ | ⇑ |
| **q** | 4.933 | 4.414 | β-Man-(1→2)-β-**Man**-(1→2)-β-Man-(1→2)-α-Man-(1→<br>β-Man-(1→2)-β-**Man**-(1→2)-β -Man-(1→2)-α-Man-(1→$PO_4$ | ⇓ | ⇓ | ⇓ | ⇓ | ⟺ | ⇓ |
| **q** | 4.930 | 4.163 | β-**Man**-(1→2)-β-Man-(1→2)-β-Man-(1→2)-α-Man-(1→$PO_4$ | ⇓ | ⇓ | ⇓ | ⇓ | ⟺ | ⇓ |
| **q** | 4.927 | 4.016 | α-**Man**-(1→6) | —[g] | —[g] | —[g] | —[g] | —[e] | —[e] |
| **r** | 4.917 | 4.309 | β-Man-(1→2)-[β-Man-(1→)]$_n$-β-**Man**-(1→2)-α-Man-(1→$PO_4$ | ⟺ | ⟺ | ⇓ | ⟺ | ⟺ | ⇓ |
| **s** | 4.904 | 4.293 | β-Man-(1→2)-[β-Man-(1→)]$_n$-β-**Man**-(1→2)-α-Man-(1→$PO_4$ | = | = | ⟺ | ⇑ | ⟺ | ⟺ |
| **t** | 4.866 | 4.276 | β-Man-(1→2)-β-**Man**-(1→2)-α-Man-(1→ | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **t** | 4.862 | 4.168 | β-**Man**-(1→2)-β-Man-(1→2)-α-Man-(1→$PO_4$ | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **t** | 4.857 | 4.168 | β-**Man**-(1→2)-β-Man-(1→2)-α-Man-(1→2(3))<br>β-**Man**-(1→2)-β-Man-(1→2)-α-Man-(1→$PO_4$ | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |
| **u** | 4.840 | 4.088 | β-**Man**-(1→2)-α-Man-(1→$PO_4$ | ⇓ | ⇓ | ⟺ | = | ⇑ | ⇑ |
| **v** | 4.791 | 4.056 | β-**Man**-(1→2)-α-Man-(1→ | ⇓ | ⇓ | ⇓ | ⇓ | ⇑ | ⇑ |

[a] Growth temperatures are shown as follows: growth temperature during three passages, slash separator, then final growth temperature.
[b] Relative to presence of the structural feature in mannan isolated from YPD with the resonance **n** for α-Man-(1→2) at 5.07 ppm set to the same height for both spectra.
[c] Symbols: = : no difference; ⟺ : very slight difference; ⇑: increase; ⇓: decrease.
[d] Repeat unit along the backbone with different side chains attached.
[e] COSY crosspeak not observed.
[f] Chemical shift for the anomeric proton of β–Man next to terminal β–Man-(1→.
[g] See text for discussion.