ß (1-3)-Glucan Unmasking in *C. auris* for Recognition by Innate Immune Cells

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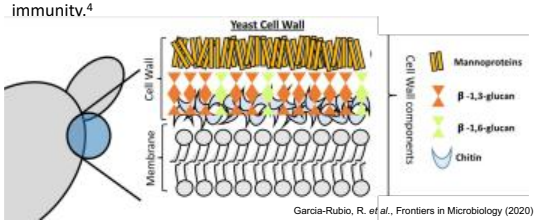
Introduction

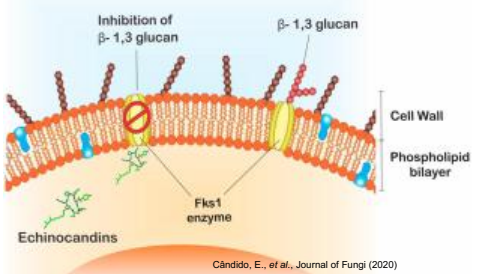
- The *Candida* genus causes superficial and systemic candidiasis, which can lead to high rates of morbidity and mortality, especially in hospital settings where it is becoming resistant to many antifungal treatments.[1]
- The first described case occurred in 2009 in Japan. Between 2013 and 2016, seven cases were identified in the United States, with the CDC issuing a clinical alert in June 2016.[2] Human infection has occurred on every continent, but extensive transmission has occurred in South Asia, America, and Africa.[3]
- The innate immune system controls *Candida* infection, through the germline-encoded β-glucan receptor Dectin-1. The cell wall unmasking has been shown to help increase the recognition of *C. albicans* by allowing Dectin-1 to recognize β-1,3-glucan and mediate immunity.[4]



Garcia-Rubio, R. *et al.*, Frontiers in Microbiology (2020)

Figure 1. Cell wall structure of *C. auris*[5]

- This means that the higher the unmasking and exposure of the β-1,3-glucan layer, the more macrophages can recognize them as antigens and trigger the appropriate immune response.[4]
- Echinocandins, specifically caspofungin, have been shown to exhibit antimicrobial tendencies in *C. auris* by specifically and non-competitively inhibiting synthesis of (1,3)-β-d-glucan.[6]



Cândido, E., *et al.*, Journal of Fungi (2020)

Figure 2. Echinocandin inhibition of β-1,3-glucan synthesis in *C. auris*[7]

Hypothesis

- We hypothesize that using sublethal levels of caspofungin will not kill the cell but expose the (1,3)-β-d-glucan layer, allowing cells to be faster recognized for the immune systems attacks.

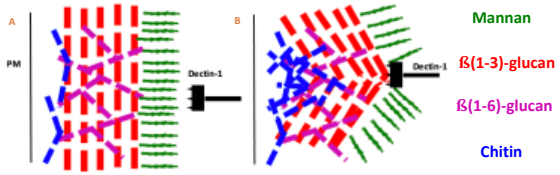
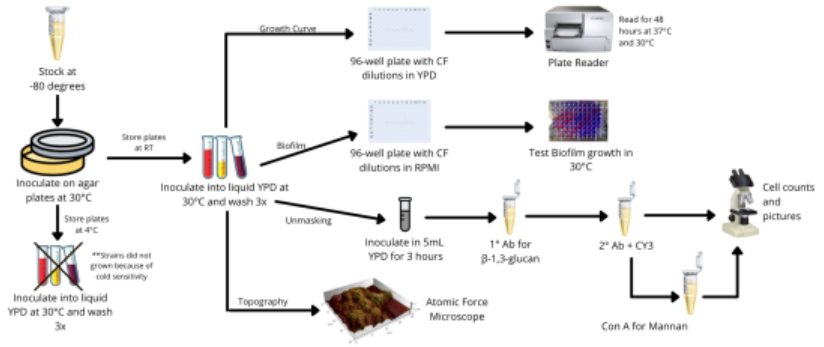


Figure 3. (A) Model for Masking: Outer mannan interferes with Dectin- 1 recognition of β-1,3-glucan. **(B) Model for Unmasking:** Damaging the mannan layer directly or increasing chitin deposition can allow Dectin-1 to bind to β-1,3-glucan and initiate signaling.[4]

Abstract

Multidrug-resistant *Candida auris*, a major hospital-acquired pathogen, is a severe health threat and poses a significant challenge to healthcare providers. Although there have been several studies on the antifungal resistance of this species, there have been very limited cell wall studies on immune responses to unmasking. *C. auris* cell walls are made of chitin and ß (1-3)-glucan, masked with a layer of mannosylated glycoproteins. This masking decreases the efficiency of immune detection of the ß (1-3)-glucan by innate immune cells (Dectin-1). Caspofungin, of the echinocandin class, has been shown to exhibit antifungal tendencies and reduce biofilm formation in *Candida* species by inhibiting the synthesis of β-(1,3)-D-glucan. We conducted a comparative unmasking study on nine clinical *C. auris* strains and one *Candida albicans* wild type (used as the control strain) with different caspofungin concentrations for the unmasking of ß (1-3)-glucan.

Methods



Data

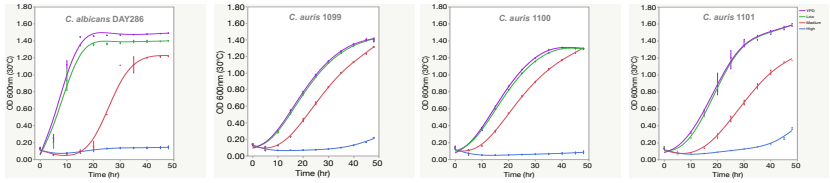


Figure 4. Growth Curve (OD600nm). Analyzed every 5 hours for 48 hours at 30°C. Mean ± SD. High concentration (100 ng/mL) showed inhibition of strain, medium concentration (50 ng/mL) showed MIC values, low concentrations (6 ng/mL) showed resistance. *C. auris* showed more resistance at medium concentrations than *C. albicans*.

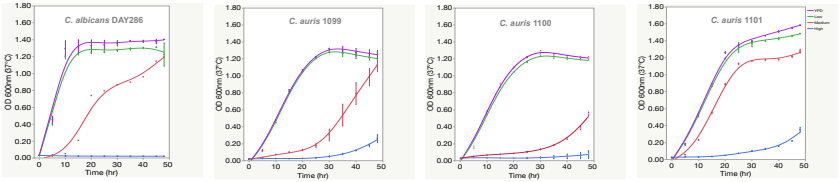


Figure 5. Growth Curve (OD600nm). Analyzed every 5 hours for 48 hours at 37°C. Mean ± SD. High concentration (100 ng/mL) showed inhibition of strain, medium concentration (50 ng/mL) showed MIC values, low concentrations (6 ng/mL) showed resistance. *C. auris* showed more resistance at medium concentrations than *C. albicans*.

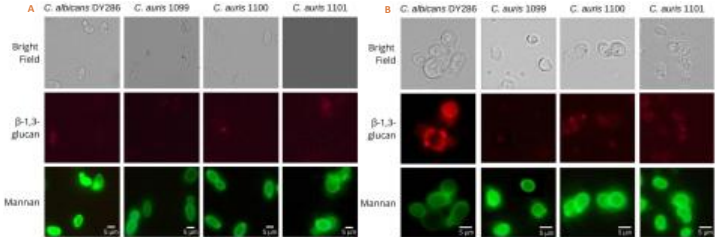


Figure 6. Fluorescent Microscope Images of (A) strains without caspofungin (50% intensity) **(B) strains with 50 ng/mL caspofungin** (90% intensity). Cells were stained with β-(1,3)-glucan 1° and Cy3-conjugated 2° antibodies and concanavalin A and viewed by immunofluorescence microscopy.

Analysis

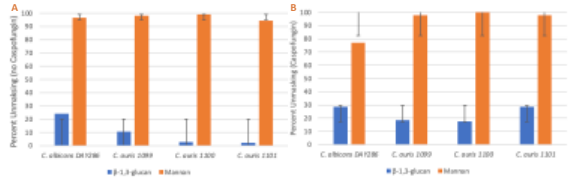
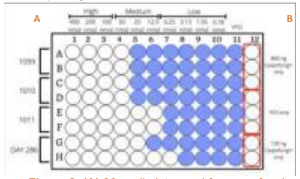


Figure 7. (A) Percent Unmasking without caspofungin. **(B)** Percentage unmasking with 50 ng/mL caspofungin.



Strains	MIC (ng/mL)
1099	37.5
1100	37.5
1101	9.375
DAY286	11.71

Figure 8. (A) 96- well plate used for caspofungin susceptibility testing in *C. auris* **(B) Minimum Inhibitory Concentration** (Concentration before growth + Concentration where grow begins)/2.

Conclusion

- All strains exhibited low to no growth at high caspofungin concentrations. MIC values show medium levels of caspofungin allowed the cell to grow without killing the strains.
- *C. albicans* showed greatest unmasking of the four strains. *C. auris* 1101 has greater, but not significant, unmasking of the three clinical strains.
- Some β- (1-3)-glucan unmasking, but this will be further explored.

Future Studies

- Additional iterations of growth curves need to be conducted with all 21 clinical strains with different concentrations of caspofungin and fluconazole. Percent unmasking also needs to be determined under each drug condition.
- In addition, we plan on watching germ tube formation to determine the composition of the biofilm as yeast or pseudohyphae, which indicates how well biofilm adheres to different surfaces.
- We also plan on testing the virulence of *C. auris* in Galleria mellonella infection.
- Finally, we plan to look at the nano level structure of the cell wall using atomic force microscope.

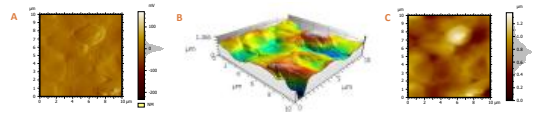


Figure 9. *C. auris* 1100 topography

Acknowledgements:

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