MNN2 Gene Affects Drug Resistance In *Candida glabrata's* Biofilms

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Candida glabrata infections

Infections caused by *Candida* species have increased worldwide substantially over the latest decades, and are a significant cause of morbidity and mortality, mostly among critically ill patients. *Candida glabrata* is the second most common *Candida* responsible for these infections in the USA and the third in Europe, and is characterized by a high antifungal resistance.

Goal of the Study

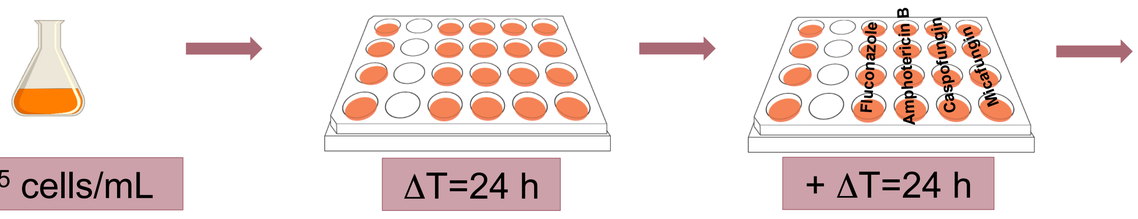
- To understand the role of mannans in *C. glabrata* biofilms and in biofilm cells resistance to antifungal drugs (fluconazole - Flu, amphotericin B - AmB, caspofungin - Csf and micafungin - Mcf).

Methods

ΔT=24 h pre-formed biofilms

A. Susceptibility of the biofilm to the antifungal agents

B. Biofilm analysis



10^5 cells/mL → ΔT=24 h → + ΔT=24 h

***C. glabrata* strains**:

C. glabrata ATCC2001 (wild-type)

C. glabrata HT6 (parent)

C. glabrata Δ*mnn2* (mutant)

Change half medium and add:

a. RPMI-1640 (control group)

b. Antifungal: Flu, AmB, Csf or Mcf*

(*at MBECs concentrations)

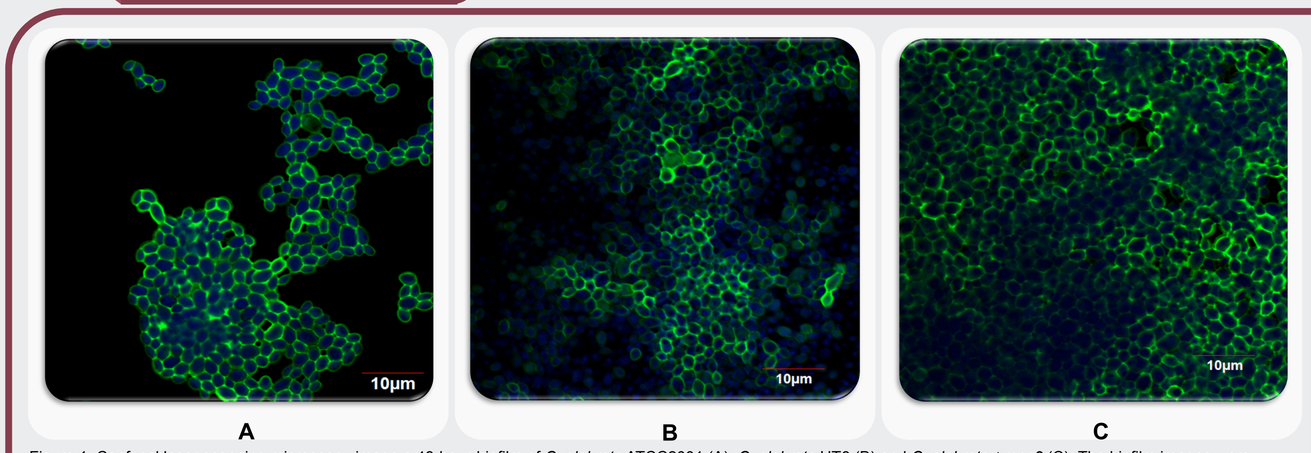
1. Biofilm structure:

- Confocal microscopy

2. Biofilm matrix analysis:

- Biomass reduction (Crystal Violet)
- Mannans (Quantitative Alcian Blue Binding Assay)
- Polysaccharides (Dubois method)
- β-1,3 glucans (Glucatell® Kit)

Results



A B C

Figure 1. Confocal laser scanning microscopy image a 48-hour-biofilm of *C. glabrata* ATCC2001 (A), *C. glabrata* HT6 (B) and *C. glabrata* Δ*mnn2* (C). The biofilm images were acquired using a Confocal Scanning Laser Microscope (Olympus BX61, Model FluoView 1000). Filters: DAPI (100 mg/L emissions filters BA 430–470) and Concavalin A, Alexa Fluor 488 conjugate (50 mg/L emissions filters BA 505–605). Images were acquired with the program FV10-ASW 4.2 (Olympus) using a magnification of 100xm Measure bar: 10 µm.

No variations on the cell wall were noticed among the strains

But… the mutant and parent strains showed a higher amount of multilayer structures, than the reference strain

Table 2. Percentages of biomass reduction of the biofilm matrices of *C. glabrata* ATCC2001, *C. glabrata* HT6 and *C. glabrata* Δ*mnn2* after ddding the drugs and comparing to the control group (0 mg/L).

% Biomass Reduction	Flu 1250	AmB 4	Csf 3	Mcf 17
C. glabrata ATCC2001	16%	52%	83%	57%
C. glabrata HT6	49%	63%	60%	64%
C. glabrata Δ*mnn2*	**67%**	**78%**	**75%**	**82%**

The lack of mannans leads to a more fragile biofilm a higher biomass loss.

***C. glabrata* Δ*mnn2* presented the top biomass reduction, specially when in contact with echinocandins.**

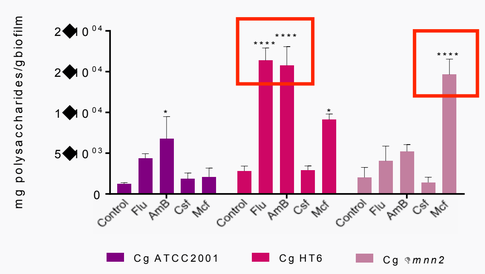


Figure 2. Polysaccharides content on the biofilm matrices of *C. glabrata* ATCC2001, *C. glabrata* HT6 and *C. glabrata* Δ*mnn2*. *(* P<0.05; **** P< 0.0001). (Cg – C. glabrata)*

Polysaccharides tended to increase in the biofilm matrices of the strains in contact with all drugs, specially with Flu, AmB for *C. glabrata* HT6 and Mcf for *C. glabrata* Δ*mnn2* (*P*>0,0001).

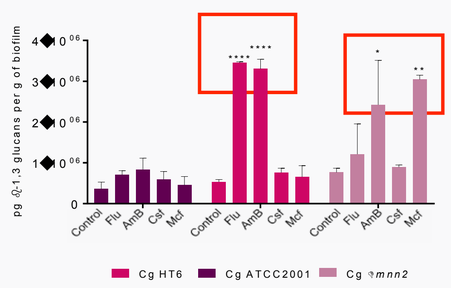


Figure 4. β-1,3 glucans concentration on the biofilm matrices of *C. glabrata* ATCC2001, *C. glabrata* HT6 and *C. glabrata* Δ*mnn2*. *(Cg – C. glabrata) (* P<0.05; ** P<0.001; *** P<0.0005; **** P< 0.0001).*

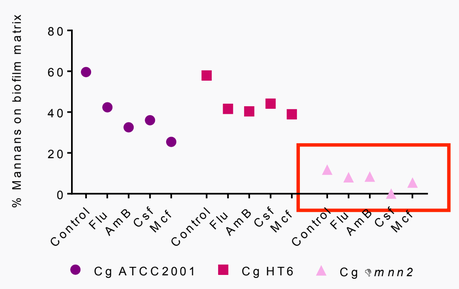


Figure 3. Alcian Blue binding assay in biofilm matrices of *C. glabrata* ATCC2001, *C. glabrata* HT6 and *C. glabrata* Δ*mnn2*. Data represent the percentage of amount of dye bound per biomass. *(Cg – C. glabrata)*

The biofilm matrices showed to reduce their mannans content in the presence of all drugs in *C. glabrata* ATCC2001 and *C. glabrata* HT6.

Interestingly, in *C. glabrata* Δ*mnn2* , these compounds were unable to be detected in the biofilm cell walls, in all conditions.

β-1,3 glucans increased in the biofilm matrices of the strains in contact with all drugs, when compared to the control group.

***C. glabrata* HT6 and the mutant, *C. glabrata* Δ*mnn2* showed to have the highest amounts in these sugars.**

Conclusion

- The KO of the *MNN2* gene does not reveals any microscopic changes in the cell wall;
- The lack of mannans leads to a more fragile biofilm and a higher biomass loss after a drug stress;
- The polysaccharides content increase in the biofilm matrix of *C. glabrata* strains in contact with Flu, AmB and Mcf, but the mannans have the opposite behavior;
- All the strains produce high quantitites of β-1,3 glucans when in the presence of all drugs, specially the mutant, which is probably attempting to compensate the lack of mannans in the matrix;
- *C. glabrata* Δ*mnn2* has a more fragile biofilm than the other strains, which can alter its drug resistance.

Acknowledgements

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