Portrait Of Gene Expression In *C. glabrata* With Stress Induced By Drugs

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Candida glabrata Biofilms

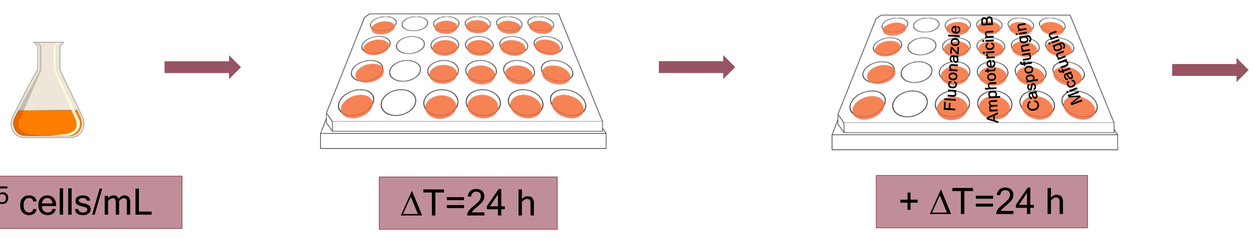
Candidiasis have globally increased over the last years, being a major cause of morbidity and mortality, especially in immunosuppressed and hospitalized patients. *Candida albicans* remains to be the most common species responsible in candidiasis, but *Candida glabrata* has appeared as second most common *Candida* in the USA and the third in Europe. Biofilms of this species are extremely difficult to eradicate and are a source of many recalcitrant infections.

Goal of the Study

- To evaluate how *C. glabrata* cells try to adjust their biofilm composition in response to an antifungal drug treatment based on a series of profiles of eight genes' expression - ***BGL2, FKS1, FKS2, GAS2, KHN1, UPG1, XOG1*** **and** ***MNN2*** - known to be related to the production of β-1,3, β-1,6-glucans and mannans.

Methods

1. Biofilm cells recovery



10^5 cells/mL → ΔT=24 h → + ΔT=24 h

***C. glabrata* strains**:

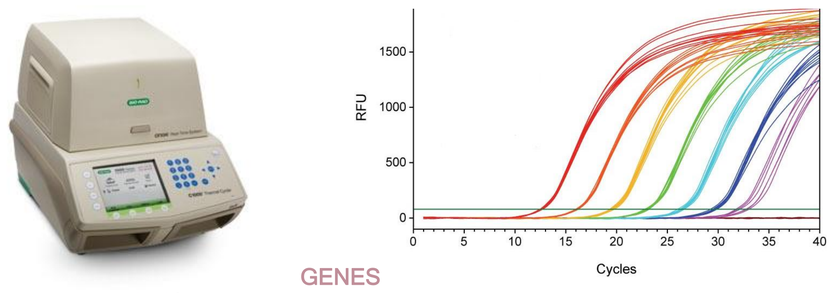
C. glabrata ATCC2001 (wild-type)
C. glabrata 562123 (urinary tract)
C. glabrata 534784 (vaginal tract)

Change half medium and add:

a. RPMI-1640 (control group)
b. Antifungal: Flu, AmB, Csf or Mcf*

(*at MBECs concentrations)

2. Real Time-qPCR Analysis



GENES

BGL2, *FKS1*, *FKS2*, *GAS2*, *KHN1*, *UPG1* - β-1,3 glucans regulation
XOG1 - β-1,3 and β-1,6 glucans regulation
MNN2 - Mannans regulation

$2^{\Delta CT}$ analysis

Results

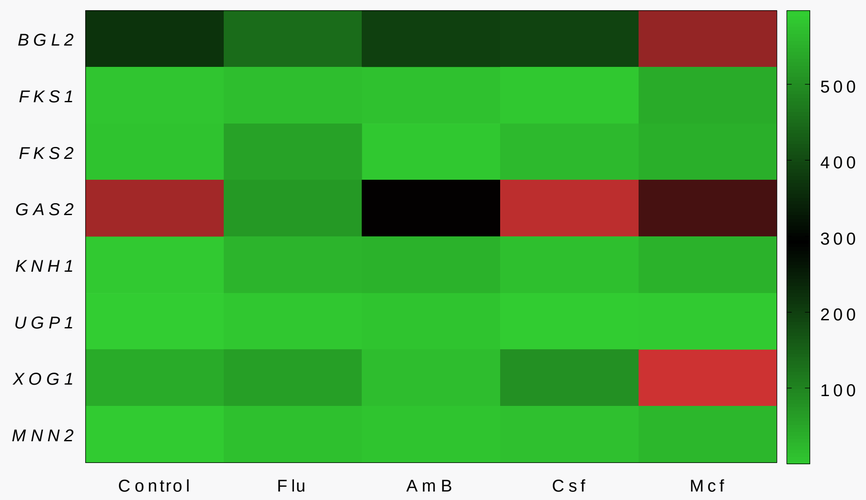


Figure 1. Real-time PCR expression profiling of biofilm cells of *C. glabrata* ATCC2001 with and without antifungal contact (MBEC concentrations). The heatmap was generated by a log transformation of the real-time PCR data treated as $2^{\Delta CT}$ and then presented as percentage of expression. (Flu: fluconazole; AmB: amphotericin B ; Csf: caspofungin; Mcf: micafungin.)

C. glabrata ATCC2001

- Overexpressed mostly *BGL2, GAS2* , *XOG1* genes;
- Mcf was responsible for the indution of the highest overexpressions.

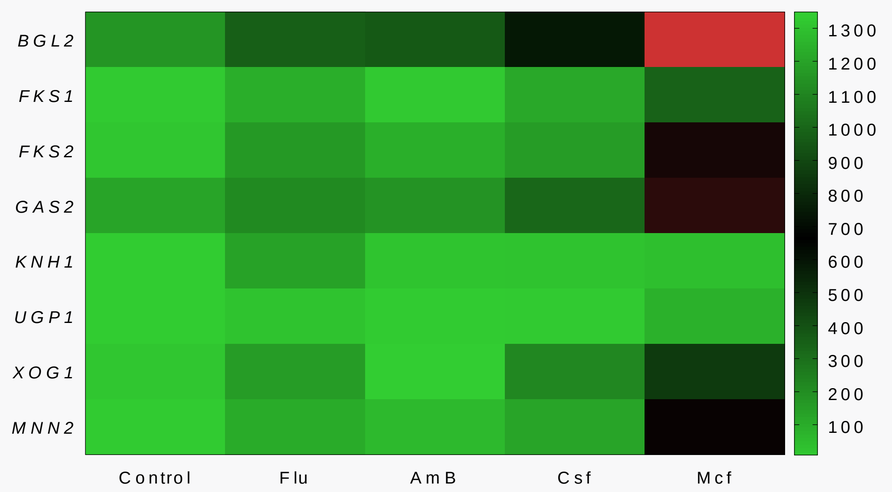


Figure 1. Real-time PCR expression profiling of biofilm cells of *C. glabrata* 534784 with and without antifungal contact (MBEC concentrations). The heatmap was generated by a log transformation of the real-time PCR data treated as $2^{\Delta CT}$ and then presented as percentage of expression. (Flu: fluconazole; AmB: amphotericin B ; Csf: caspofungin; Mcf: micafungin.)

C. glabrata 534784

- Higher capacity to rapidly alter the genes expression after a stress induced by any of the drugs;
- *BGL2, GAS2, FKS1, FKS2 and XOG1* : changes, especially when in the presence of Flu and both echinocandins;
- Mcf: responsible for the highest variations in the genes expressions.

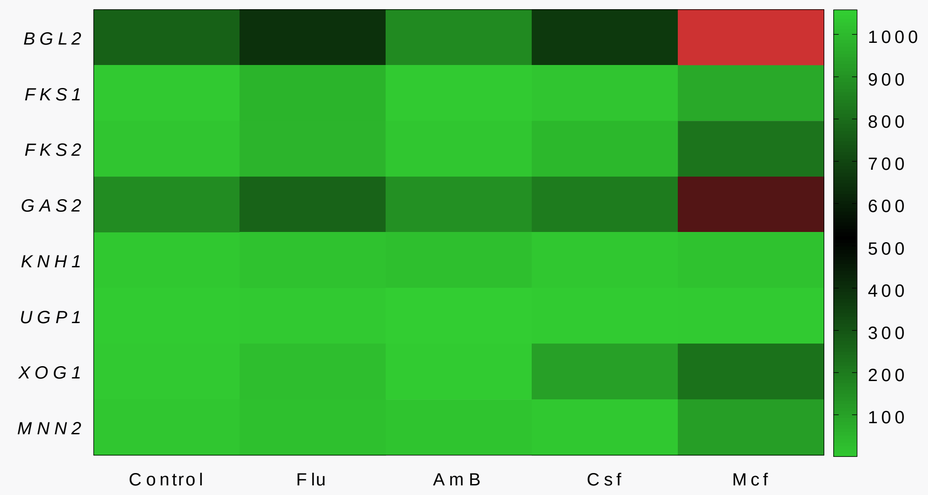


Figure 1. Real-time PCR expression profiling of biofilm cells of *C. glabrata* 532123 with and without antifungal contact (MBEC concentrations). The heatmap was generated by a log transformation of the real-time PCR data treated as $2^{\Delta CT}$ and then presented as percentage of expression. (Flu: fluconazole; AmB: amphotericin B ; Csf: caspofungin; Mcf: micafungin.)

C. glabrata 562123

- Overexpressed *BGL2, GAS2* and *XOG1* genes, in presence of most drugs, but in a much higher magnitude than *C. glabrata* ATCC2001;
- Mcf was responsible for overexpressing 6 of the 8 analysed genes, except *UPG1* and *KHN1* genes.

Conclusion

- The gene expression profiles were dependent on the strain, gene and drug, revealing high *Candida glabrata* intra-strains variations;
- As it is known, β-1,3 glucans are important means of matrix protection to the *Candida* biofilm cells. The overexpression of the genes, related to their regulation, showed an attempt of the cells to increase glucans production for the biofilm matrices, in order to protect them against the drugs pressure;
- This work demonstrates the plasticity of biofilm cells and the high capacity of *C. glabrata* cells to adapt and respond properly to any antifungal drug aggression, which can explain, to some degree, the particular high virulence associated to this species.

Acknowledgements

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