Global transcriptome characterization of *Candida glabrata* biofilms in response to acetate and fluconazole

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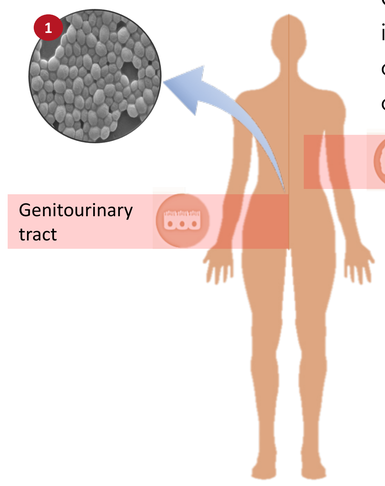
1. INTRODUCTION

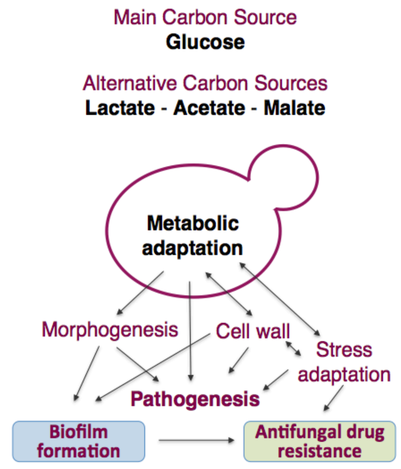


DEADLY, DRUG-RESISTANT *CANDIDA* INFECTIONS

Candida glabrata thrives as a normal flora in healthy individuals but when immune defenses are compromised it is responsible for life-threatening disseminated infections.

1. This fungus uses the **biofilm lifestyle** as a **mode of protection** which facilitates survival in diverse **environmental niches.**
2. In order to adapt and survive in these different environmental niches, *C. glabrata* assimilates **alternative carbon sources** such as acetate.

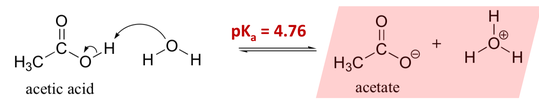
ADAPTATION TO COMPLEX HOST-NICHES



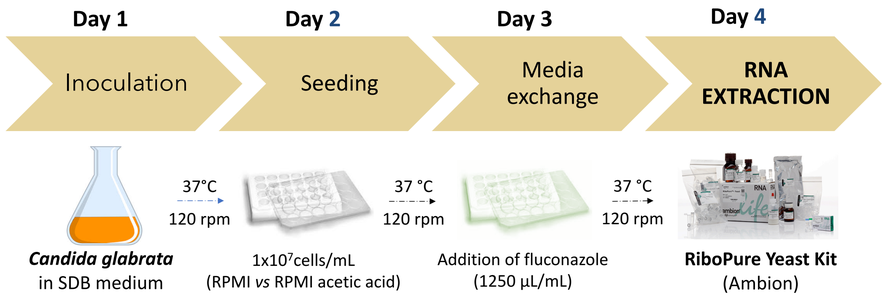
2. AIM

To identify the molecular mechanisms in ***C. glabrata*** that contribute to **biofilm formation** and **antifungal drug resistance**, in acidic pH niches associated with the presence of acetic acid, following a RNA-Seq approach.

Acetate as an alternative carbon source

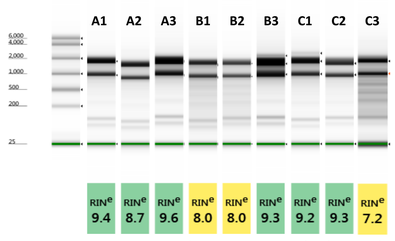


3. METHODOLOGY

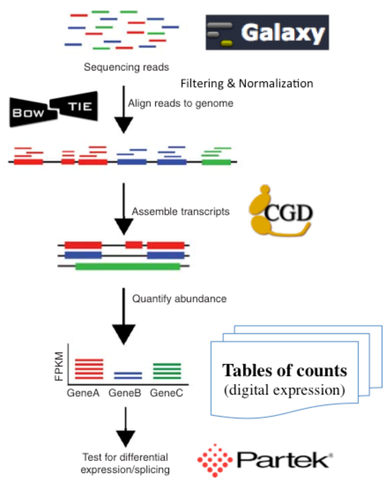


RNA QUALITY CONTROL AND SEQUENCING

- RNA quality was assessed on a **Bioanalyzer 2100**.
- Library preparation and sequencing of RNA was performed at **Edinburgh Genomics** (Scotland, UK).
- All samples were prepared in triplicate and subjected to removal of rRNA before cDNA library generation.
- 50 base single-end sequence reads were produced with **Illumina Hiseq 2000** from cDNA libraries.



A. RPMI; **B.** RPMI acetic acid; **C.** RPMI acetic acid + Fluconazole



BIOINFORMATIC DATA ANALYSIS

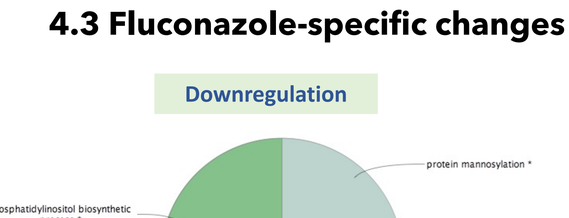
- Raw fastq files were processed in the following order: **Fastqc** (v.10.1), **Trimgalore** (v.3.1), **Samtools** (v.1.19), **Bowtie2** (v.2.1) and **Htseq** (v.5.4).
- Genome alignment was conducted against the *C. glabrata* genome file provided by **CGD.**
- Gene expression analysis was performed using **Partek** software (v. 6.6).
- GO-term analysis was performed in parallel through the **CGD GO Term Finder** and the **Cytoscape** (v. 3) Clue GO plugin.
- Venn diagrams were performed with **Venny** (v. 2.0.2) and heat maps with **TM4 MultiExperiment Viewer** (MeV; v. 4.9).

QUANTITATIVE REAL-TIME PCR (qPCR)

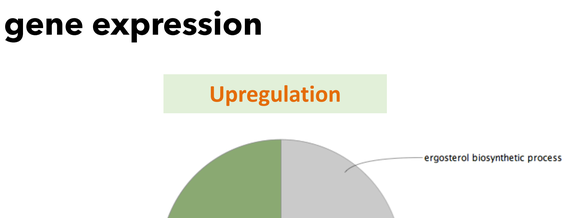
- Gene expression was calculated using the **comparative C_T method** (PGK1 used as housekeeping gene).

4. RESULTS

4.1 Differentially expressed genes between the different conditions

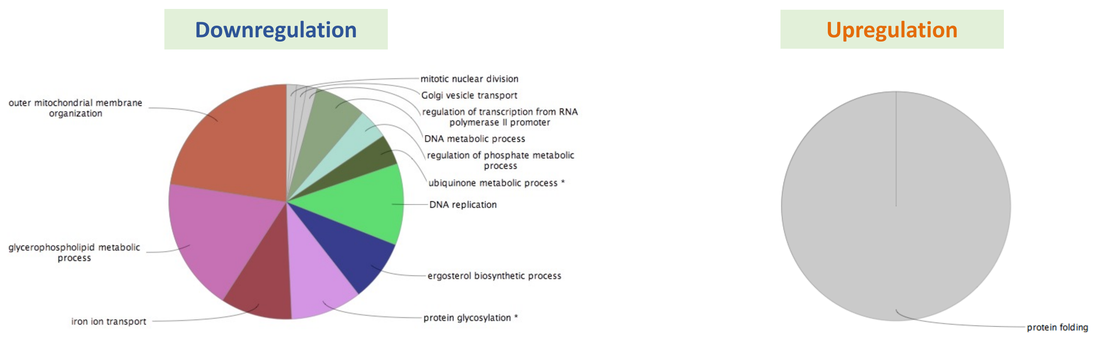


(P < 0.005; cut-off≥-2-fold change)



(P < 0.005; cut-off≥2-fold change)

4.2 Acetate-specific changes in gene expression

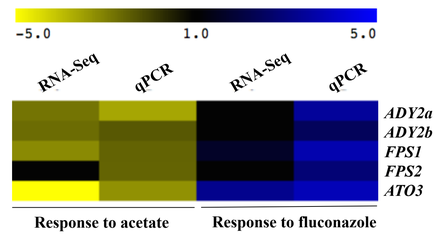


4.3 Fluconazole-specific changes in gene expression

4.4 Validation of RNA-seq results

To validate the findings from our RNA-Seq analysis, we performed qPCR for 5 putative acetate transporters: ***ADY2a***, ***ADY2b***, ***FPS1***, ***FPS2*** and ***ATO3***.

qPCR results are in agreement with the results from the RNA-Seq analysis.



5. CONCLUSIONS

- ✓ Adaptation of biofilms to the alternative carbon source acetate is achieved by a considerably downregulation of various metabolic processes.
- ✓ Dissecting the RNA-seq data allowed us to recover the essential pathway biology behind fluconazole resistance in biofilms formed in the presence of acetate.
- ✓ Understanding and targeting some of these pathways is potentially useful for developing diagnostics and new antifungals to treat biofilm-based infections.

6. ACKNOWLEDGMENTS

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