Evolution of *Candida albicans* posaconazole tolerance

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INTRODUCTION

Candida albicans is a commensal member of the human microbiome, but overgrowth can lead to Candidiasis – a fungal infection that can occur in the mouth, vagina, or bloodstream[1].

Resistance to azoles, a common anti-fungal used to treat infections, has been well-documented – particularly for fluconazole[2]. These include mutations in *ERG11*[3], *RTA2*, and up-regulation of *CDR1* and *CDR2*[4]. However, for the newer azole posaconazole, genetic mechanisms for resistance are less well-documented.

This project sought to enact a selective pressure on various strains of *C. albicans,* with the goal of evolving posaconazole tolerance or resistance. Drug resistance refers to a measurable increase in the minimum concentration required to inhibit growth, while drug tolerance (see Figure 1) can be defined as the proportion of a population capable of slow growth at concentrations *above* the minimum inhibitory concentration[5]. Evolved strains were characterized both phenotypically and genotypically to identify a genetic basis for posaconazole tolerance.

MATERIALS AND METHODS

EVOLUTION

- Eight strains of *C. albicans* (12 replicates per strain; 96 total replicates) were grown in medium containing YPD + 0.5 µg/mL posaconazole
- The cell population was diluted 1000-fold every 72 hours and transferred to fresh medium
- After five transfers (~ 100 generations), the evolved populations were frozen as glycerol stocks for phenotyping and genotyping

DISK DIFFUSION ASSAY

- Evolved and ancestral replicates were grown on solid YPD media in the presence of a disk with 2.5mg posaconazole
- Plates were photographed after 24 and 48 h and analyzed computationally using the diskImageR[6] R package to measure tolerance and resistance

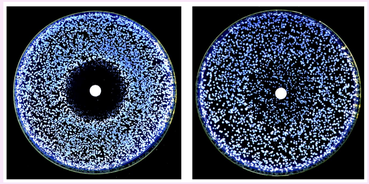

Figure 1. Ancestral (left) and posaconazole-evolved (right) *C. albicans* strain SC5314 growing on YPD media in the presence of a posaconazole-containing disk. The evolved strain has increased in tolerance, with a large fraction of growth occurring within the zone of inhibition.

WHOLE GENOME SEQUENCING

- Genomic DNA from strain SC5314 was sequenced using the Illumina NextSeq 550 platform to a calculated depth of ~40 by the MiGS Sequencing Center (Pittsburgh, USA)

RESULTS - EVOLUTION

All strains exhibited a decrease in biomass production (OD at the time of transfer) after the initial transfer into posaconazole-containing media. After the initial transfer, two general trends were observed: either the majority of replicates went extinct (top panels, figure 2) or there was some recovery of biomass production by the time of the fifth transfer (bottom panels).

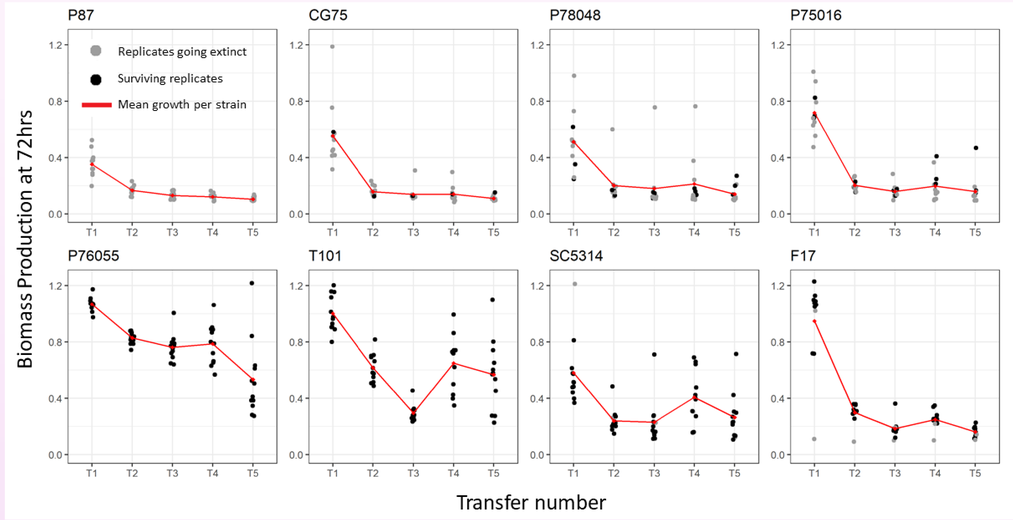

Figure 2. Growth ability of various strains of *C. albicans* over five subsequent transfers into YPD media supplemented with 0.5µL posaconazole. Biomass was measured via spectrophotometer as optical density as 600nm at the time of transfer.

RESULTS - PHENOTYPE

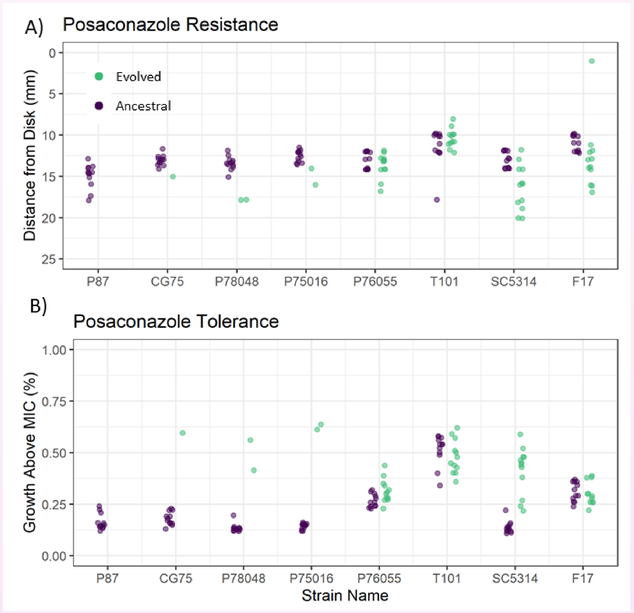

Figure 3. Comparison between ancestral and posaconazole-evolved strains of *C. albicans*. A) Resistance was determined as the radius from the disk at which there was a 20% reduction in growth ability. B) Tolerance was determined as the fraction of the population growing within the zone of inhibition determined from A).

While no notable increases in resistance were observed, most evolved strains displayed observable increases in tolerance compared to the ancestral strains. As a general trend, strains with the lowest ancestral tolerance levels displayed the greatest improvement.

RESULTS - GENOTYPE

QC
- Quality of reads checked with **FASTQC**[7] before and after trimming
- **Trimmomatic**[8] used to remove adaptors, low quality reads, and very short reads

Alignment
- **BWA**[9] used to align reads to SC5314 reference genome

Variant Calling
- SNPs identified with **GATK**[10]
- Results visualized with **IGV**[11]

Table 1. Putative genes of interest for posaconazole tolerance. Genes were selected that met the following criteria: mutations present in the evolved strains but not in the ancestral strains, mutations had to be observed in a minimum of five other strains, and the gene must have a known association with azole drugs.

Gene Name	Notes*	Position	Mutation	# of Strains
IFD6	Aldo-keto reductase family member; increased protein correlates with MDR1 overexpression in fluconazole-resistant clinical isolates	chr1:867820 chr1:867829	G/G → G/A G/G → G/A	7 5
PNG2	Putative peptide:n-glycanase; transcription up-regulated by treatment with caspofungin, ciclopirox olamine, ketoconazole, or hypoxia	chr2:1158412	A/A → A/G A/A → G/G	3 10
ALS2	ALS family protein; role in adhesion, biofilm formation induced by ketoconazole, low iron and at cell wall regeneration	chr6:977291 chr6:977293 chr6:977294 chr6:977306	C/C → C/T C/C → C/A A/A → A/T A/A → A/C	7 7 7 7
ALS3	ALS family cell wall adhesin; role in epithelial adhesion; fluconazole-repressed	chrR:1533684 chrR:1533687 chrR:1533705 chrR:1533708 chrR:1533732 chrR:1533750 chrR:1533751	T/T → T/A A/A → A/T G/G → G/A A/A → A/G T/T → T/C T/T → T/G G/G → G/T	7 7 7 7 7 6 6
IFC3	Oligopeptide transporter; fluconazole-induced	chrR:499325 – 494327 chrR:494320	2bp del 2bp ins	10 10

CONCLUSIONS

Drug tolerance and drug resistance are separate and distinct traits that can be evolved independently of one another. Strains with lower initial tolerance display the greatest increase in tolerance.

While there were no differences observed between the ancestral and evolved SC5314 samples in genes known to be associated with azole resistance[4], novel mutations in genes known to be transcriptionally induced by azole drugs were observed. These genes may be involved in posaconazole tolerance.

FUTURE DIRECTIONS

- Test the effect of an increased number of transfers or increasing the interval between transfers. Does the level of tolerance continue to evolve with additional transfers? Do resistance mechanisms evolve, and if so, how many transfers are required for this?
- Does the acquisition of posaconazole tolerance result in the acquisition of tolerance to other azole drugs?

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