**Chapter 1**

**Evaluation of *C. glabrata* growth, treated with antifungal drug under different environmental conditions**

**Introduction**

Antifungal drugs mount selective pressure on the organisms, leading to survival of only those cells that acquire resistance. However, currently available antifungal drugs are mainly fungistatic rather than fungicidal empowering the pathogenic fungal cells to quickly adapt to hostile environments by few genetic modification and improved genome plasticity. The fungistatic nature of these drugs initiates strong directional selection for evolution of resistant strains (Pais et al., 2019).

Frequent use of azoles as prophylactic agent in high-risk patients have significantly risen antifungal drug resistance in *C. glabrata* but not in other Candida species (Wiederhold, 2017). Gain of function mutation in transcription regulator PDR1 induces expression of drug efflux pump (Cdr1, Cdr2, Snq2 and Qdr2), confers antifungal drug resistance and enhances virulence in *C. glabrata* is a widely discussed mechanism (Sanguinetti et al., 2005; Vermitsky and Edlind, 2004). Additionally, mitochondrial dysfunctional has been linked with drug resistance by promoting expression of PDR1 (Defontaine et al., 1999; Ferrari et al., 2011). Another study have illustrated role of calcium signaling in azole resistance as calcium depletion switches fluconazole from fungistatic to fungicidal (Kaur et al., 2004). These and many other studies (Ferrari et al., 2009; Salazar et al., 2018) highlight core mechanism underlying antifungal resistance and virulence, however how the mechanisms evolved with respect to each environment is poorly investigated. Here we attempt to elucidate the effect of different environmental condition on *C. glabrata,* to adapt and resist antifungal drugs.

**Results**

1. **Oxidative stress treated *C. glabrata* cells elevated resistance to fluconazole**

Upon engulfment by host immune cells like macrophages and neutrophils *C. glabrata* cells are continuously bombarded by reactive oxygen species inducing oxidative stress response in fungi. To understand the oxidative stress response, we first studied the whole-genome transcriptional profile of *C. glabrata* cells treated with hydrogen peroxide (H2O2). Our data showed that prolonged oxidative stress induces expression of genes involved in ergosterol biosynthesis pathway, which is target of azoles (Pais et al., 2019). This points to the hypothesis, cells pre-exposed to oxidative stress are resistant to antifungal drug.

To study the effect of antifungal drug on *C. glabrata* cells and more specifically cells with oxidative stress experience, we monitored fungal growth in presence of antifungal drug. Single *C. glabrata* colony was allowed to grow overnight and a population of 0.1 OD600 cells were allowed to grow in fresh media (YPD) for log-phase in two flasks for 4 hours. Cells were then allowed to grow for another 4 hours with one flask treated with 20 mM H2O2 while equivalent amount of water was added in another, referred as control. Once the cells get adapted to oxidative environment, they were further diluted to 0.01 OD600 in fresh media. These cells were then treated with antifungal drug, fluconazole. From clinical studies, it is been observed that patients with azole treatment show fluconazole MIC (Minimum Inhibitory Concentration) 32ug/ml while non-azole treated group have MIC 64ug/ml for *C. glabrata* (Eschenauer et al., 2018; Ko et al., 2018)*.* Since *C. glabrata* shows a broad range of inhibitory concentration we tested effect of fluconazole over the range of 16 to 64 ug/ml. Post fluconazole treatment, fungal cells were aliquoted in 96 well-plate and growth was monitored every half hour using cytation3 Figure 2.1. illustrates schematic representation of methodology used in this experiment. Growth curve represents average of three wells for each of the two biological repeats.

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**Figure 2.1. Schematic diagram illustrating antifungal drug treatment post oxidative stress adaptation in *C. glabrata.***Over night grown Candida cells were allowed to adapt to oxidative stress. Adapted cells were treated with fluconazole to test the antifungal drug resistance. Using Cytation3 machine fungal growth was monitored for 24 hours.

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Other drugs were also tested but showed no effect.

1. ***C. glabrata* cells exhibits different growth profile under different growth medium**
2. **Fluconazole effect varies with glucose concentration**
3. **Cells growing in acidic pH are more resistant to Fluconazole than alkaline**
4. **Pre-exposure to Fluconazole improves resistance against higher concentration of Fluconazole**
5. **Days affect flu effect**