**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 the 0.1 OD cells were allowed to grow in fresh media for log-phase in two flasks for 4 hours. Cells were 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 treated with antifungal drug fluconazole.

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**