UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Master of Science*** *of*

IQRA RAZZAQ

on Wednesday, February 3, 2021 at 1:30 p.m. (online)

Thesis Title: **Analyzing the roles of the essential gene *TRA1* and the novel extracellular vesicle *EVP1* in regulating morphogenesis and the antifungal drug resistance response in the opportunistic fungal pathogen *Candida albicans***

Examination Committee:

Dr. Mike Emes, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Rebecca Shapiro, Dept. of Molecular and Cellular Biology
Dr. Jennifer Geddes-McAlister, Dept. of Molecular and Cellular Biology
Dr. Joseph Yankulov, Dept. of Molecular and Cellular Biology

Advisory Committee:
Dr. R. Shapiro (Advisor)
Dr. C. Khursigara
Dr. J. Geddes-McAlister

Abstract: Fungal pathogens, which have historically been understudied, are important disease-causing agents with global significance. *Candida albicans* is an opportunistic fungal pathogen and the leading cause of candidiasis, a systemic bloodstream infection with 40% mortality. Dynamic morphogenic plasticity allows *C. albicans* to evade host immune responses and rapidly adapt to new environments. Additionally, *C. albicans* possesses a diverse arsenal of adaptive mechanisms, which confer resistance to all three classifications of antifungal drugs (azoles, polyenes, and echinocandins), emphasizing the importance of exploring new targets for the development of novel antifungals. The role of the essential gene *TRA1* in *C. albicans* has not previously been studied in literature. We used CRISPR strategies to introduce three arginine to glutamine point mutations into *TRA1* (R3471Q, R3472Q, and R3538Q), which have previously been shown to confer antifungal sensitivities when introduced into the non-pathogenic yeast model *S. cereviciae*, generating a *tra1*$_{Q3}$ mutant. Phenotypic profiling of *tra1*$_{Q3}$ revealed that the mutant is highly azole resistant to fluconazole and miconazole, while subsequently demonstrating hypersensitivity to cell wall stressors caspofungin and calcofluor white, demonstrating an example of collateral sensitivities within fungi, a phenomenon previously observed only in bacteria and cancer cells. RNA-seq analysis of *tra1*$_{Q3}$ revealed 289 differentially expressed genes between *tra1*$_{Q3}$ and the WT, of which 188 were downregulated in the mutant and 101 genes were upregulated. From this data, we identified and further explored the gene *Orf19.6741*, which has not been studied in literature, and created a novel Δ*Orf19.6741* knockout mutant (Δ*evp1*), which demonstrated azole hypersensitivity. Both *tra1*$_{Q3}$ and Δ*evp1* demonstrated impaired filamentation and biofilm formation, antifungal sensitivity, and reduced macrophage evasion, indicating their potential involvement in multiple stress response pathways. Together, this work demonstrates the novel cellular roles of two previously uncharacterized genes in *C. albicans*.

Curriculum Vitae: Iqra completed her Bachelor of Science (Hons.) at the University of Guelph in the summer of 2018, and then began her MSc in the lab of Dr. Rebecca Shapiro in the fall of the same year.