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 ***Doctor of Philosophy*** *of*

VIOLA HALDER

on Friday, June 7th, 2024 at 1:00p.m. (SSC 2315)

Thesis Title: A dual approach for novel antifungal discovery through genetic interaction analysis in *Candida albicans* stress response mechanisms and chemical screening for novel antifungal potential

Examination Committee:

Dr. Jaideep Mathur, Molecular and Cellular Biology (Exam Chair)
Dr. Rebecca Shapiro, Dept. of Molecular and Cellular Biology
Dr. George van der Merwe, Dept. of Molecular and Cellular Biology
Dr. Terry Van Raay, Dept. of Molecular and Cellular Biology
Dr. Isabelle Benoit Gelber, Dept. of Biology, Concordia University (External Examiner)

Advisory Committee:
Dr. Rebecca Shapiro (Adv)
Dr. George van der Merwe
Dr. Krassimir Yankulov
Dr. Paul Spagnuolo

Abstract: *Candida albicans* is an opportunistic fungal pathogen commonly found in the human microbiome. With the ability to cause fungal infections that range from mild and superficial to severe, systemic and invasive, *C. albicans* is the fourth most common cause of nosocomial infections in the United States and the most common cause of candidiasis. Fungal stress response factors are essential in allowing *C. albicans* cells to survive during infection, and canalso modulate antifungal drug resistance, as antifungal drugs can impart stress on the fungal cell. Understanding *C. albicans* stress responses is prudent in novel antifungal drug discovery. Furthermore, given the limited arsenal of antifungal drugs available to combat *C. albicans* infections and the rapid rise in antifungal drug resistance, the need for novel antifungal discovery is also rising. In this thesis, we exploited a genetic approach and an optimized CRISPR-Cas9-based genome editing platform to map out genetic interactions between fungal stress response genes with roles in fungal cell survival. We uncovered negative interactions between characterized and uncharacterized genes, illuminating key epistatic interactions that could provide fundamental information into the functional redundancy in *C. albicans*. We discovered negative interactions between *SSN3* and *MNL1*, *RHB1* and *C2_10540W_A* (known as *MIG2*), and between *RHB1* and *C3_00570C_A*. We also focus on chemical screens, using natural compounds and antimicrobial peptides, to identify novel compounds with antifungal properties. We confirmed that glabridin possesses antifungal properties and proved that sapindoside A can inhibit *C. albicans* growth. Of particular interest, morin and gensenoside CK demonstrated previously unknown antifungal effects, indicating

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their potential as new therapeutic agents. Furthermore, antimicrobial peptides, STIP3-1, STIP3-14, STIP3-29, and STIP3-46 all strongly inhibited *C. albicans* growth. Additionally, ginsenoside CK showed synergy with fluconazole, and all peptides demonstrated synergy with caspofungin. Sapindoside A, STIP3-1, and STIP3-14 further demonstrated enhanced survival in *Caenorhabditis elegans* post-*Candida* infection. In conclusion, this research not only elucidates crucial stress response mechanisms in *C. albicans* but also unveils novel antifungal compounds, offering promising avenues for future drug development. These findings underscore the imperative need for diversifying the antifungal armamentarium to combat the escalating threat of *C. albicans* infections and antimicrobial resistance.

Curriculum Vitae: Viola obtained her Bachelor of Mathematics (Honours Combinatorics and Optimization, Joint Honours Pure Mathematics and Joint Honours Psychology; with distinction), at the University of Waterloo in 2013. She also obtained her Bachelor of Science (Honours Biomedical Sciences), at the University of Waterloo in 2017. Viola completed her Master of Biotechnology, at the University of Guelph in 2018. In the winter of 2019, she entered into the Molecular and Cellular Biology Ph.D. program under the supervision of Dr. Shapiro.

Awards:

NSERC - CGSD, January 2021 - April 2023
NSERC - PGSD, May 2020 - December 2020
Ontario Graduate Scholarship – Gregory OGS Fund, May 2019 - May 2020
EvoFunPath Travel Award, March 2023
CBS Mycology Award, July 2020
Candida and Candidiasis Microbiology Poster Prize, May 2023
Ontario Graduate Scholarship - Queen Elizabeth II Graduate Scholarship in Science and Technology - May 2020 (Declined)

Publications: Maksimoska, V., Goodall, C., **Halder, V.,** Agyare-Tabbi, M., Notarandrea-Alfonzo, J., López, D. G., ... & Szaszi, K. (2023). Characterizing a novel antimicrobial molecule effective for drug-resistant pathogens and bacterial biofilm that augments keratinocyte migration. *The Microbe*, 100032.

Razzaq, I., Berg, M.D., Jiang, Y., Genereaux, J., Uthayakumar, D., Kim, G.H., Agyare-Tabbi, M., **Halder, V.**, Brandl, C.J., Lajoie, P. and Shapiro, R.S. (2021). The SAGA and NuA4 component Tra1 regulates *Candida albicans* drug resistance and pathogenesis. *Genetics, 219(2)*, iyab131.

Gervais, N. C., **Halder, V.**, & Shapiro, R. S. (2021). A data library of *Candida albicans* functional genomic screens. *FEMS Yeast Research, 21(7),* foab060.

Halder, V., McDonnell, B., Uthayakumar, D., Usher, J., & Shapiro, R. S. (2020). Genetic interaction analysis in microbial pathogens: unravelling networks of pathogenesis, antimicrobial susceptibility and host interactions. *FEMS Microbiology Reviews.*

Halder, V., Porter, C. B., Chavez, A., & Shapiro, R. S. (2019). Design, execution, and analysis of CRISPR–Cas9-based deletions and genetic interaction networks in the fungal pathogen *Candida albicans*. *Nature protocols, 14(3)*, 955 - 975.