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*

BRIANNA BALL

on Wednesday, July 17th, 2024 at 9:30a.m. (SSC 2315)

Thesis Title: **Discovery and characterization of new strategies to combat *Cryptococcus neoformans***

Examination Committee:

Dr. Steffen Graether, Molecular and Cellular Biology (Exam Chair)
Dr. Jennifer Geddes-McAlister, Dept. of Molecular and Cellular Biology
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Matthew Sorbara, Dept. of Molecular and Cellular Biology
Dr. Marcio Rodrigues, Senior Investigator in Science, Technology, and Innovation in Public Health, Instituto Carlos Chagas – Fiocruz, Brazil (External Examiner)

Advisory Committee:

Dr. Jennifer Geddes-McAlister (Adv)
Dr. Rebecca Shapiro
Dr. Cezar Khursigara

Abstract: Combatting the increasing rate of fungal infections is a top priority to protect global health. *Cryptococcus neoformans* is an opportunistic fungal pathogen equipped with sophisticated virulence factors that modulate the host immune system and promote fungal survival in immunocompromised individuals, often resulting in fatal cases of cryptococcal meningitis. The lack of adequate antifungal treatments and the presence of drug-resistant strains motivates innovative strategies to identify and characterize novel druggable targets and investigate mechanisms licensing fungal virulence. In this Thesis, I employ mass spectrometry (MS)-based proteomics to dissect multiple facets of fungal adaptation to infection, nutrient-availability, and co-infection stress conditions combined with virulence phenome fingerprinting to define putative fungal therapeutic candidates. Herein, I profiled the infection dynamics of *C. neoformans* with primary macrophages to explore fluctuations in the fungal infectome and identified putative antifungal, antivirulence, and infection-relevant candidates. Prioritization of a fungal-conserved and virulence-associated protein, CipC, revealed novel connections to extracellular vesicles capable of achieving enhanced immunogenic protection as a vaccine platform with potential pan-fungal applications. Additionally, an Hsp90 co-chaperone, Wos2, originally identified in the fungal infectome, revealed important stress response roles during proteomic evaluation of zinc availability and featured defined roles in virulence determinant production and zinc utilization. Functional characterization of Wos2 revealed a critical role in intracellular and extracellular antioxidant protection that translated to a reduced affinity to the phagosomal lifestyle *in vitro* and attenuated virulence in a murine model of cryptococcosis. Finally, the antifungal candidate, CNAG_05997, corroborated its significant association in disease mechanisms following identification in a proteomic investigation of single- and dual-infection with the nosocomial bacterial pathogen *Klebsiella pneumoniae* and macrophages, in which proteomic elucidation was made feasible due to data-independent acquisition advancements in MS technology. Phenome fingerprinting of CNAG_05997 uncovered a novel determinant of fungal virulence with a

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complete reduction in all *C. neoformans* virulence factors and attenuated virulence in a murine model, supporting the discovery of a novel putative druggable target. Altogether, this Thesis provides new biological and mechanistic insights into the proteomic remodelling of *C. neoformans* across diverse infection-relevant stressors to elucidate novel therapeutic targets to suppress fungal pathogenicity.

Curriculum Vitae: Brianna obtained her Bachelor of Science in Biochemistry (Co-op) with a minor in Microbiology at the University of Guelph in 2018. In the fall of 2019, she entered into the MSc. program in Molecular and Cellular Biology under the supervision of Dr. Jennfier Geddes-McAlister. In the fall of 2020, she transferred into the PhD program. In the spring of 2023, she received a certificate for the Clinical & Regulatory Affairs Experience Training Program at the RI-MUHC, McGill University.

Selected Publications:

Ball B, Sukumaran A, Pladwig S, Kazi S, Chan N, Modrakova M, Geddes-McAlister, J. 2024. The cryptococcal co-chaperone, Wos2, drives proteome remodeling and fungal survival under diverse stressors and modes of infection. Microbiol Spectr. DOI: 10.1128/spectrum.00152-24

Sukumaran A* & **Ball B***, Krieger, J.R., Geddes-McAlister, J. 2022. Cross-kingdom infection of macrophages reveals pathogen- and immune-specific global reprogramming and adaptation. mBio. https://doi.org/10.1128/mbio.01687-22 *Authors contributed equally.

Liu B, Johal D, Buchanan R, **Ball B**, Serajazari M, Geddes-McAlister J. 2023. Quantitative phosphoproteome analysis of a cereal crop fungal pathogen's interaction with the host. Methods Mol Biol: Plant-Pathogen Interactions. https://link.springer.com/protocol/10.1007/978-1-0716-3159-1_13

Ball B, Krieger J, Geddes-McAlister J. 2022. Phosphoproteomic sample preparation for global phosphorylation profiling of a fungal pathogen. Methods Mol Biol: Proteomics in Systems Biology. https://doi.org/10.1007/978-1-0716-2124-0_10

Ball B, Woroszchuk E, Sukumaran A, Afaq A, Carruthers-Lay D, Gee L, Langille M, Geddes-McAlister J. 2021. Proteome and secretome profiling of zinc availability in *Cryptococcus neoformans* identifies Wos2 as a subtle influencer of fungal virulence determinants. BMC Microbiol. https://doi.org/10.1186/s12866-021-02410-z

Retanal C, **Ball B**, Geddes-McAlister J. 2021. Post-translational modifications drive success and failure of fungal-host interactions. J Fungi. https://doi.org/10.3390/jof7020124

Ball B, Sukumaran A, Geddes-McAlister J. 2020. Label-free quantitative proteomics workflow for discovery-driven host-pathogen interactions. JOVE. https://dx.doi.org/10.3791/61881

Ball B, Langille M, Geddes-McAlister. 2020. Fun(gi)OMICs: advanced and diverse technologies to explore emerging fungal pathogens and define mechanisms of antifungal-resistance. mBio. https://doi.org/10.1128/mbio.01020-20

Ball B, Geddes-McAlister J. 2019. Quantitative proteomic profiling of *Cryptococcus neoformans*. Curr Protoc Microbiol. https://doi.org/10.1002/cpmc.94

Ball B, Bermas A, Carruthers-Lay D, Geddes-McAlister J. 2019. Mass spectrometry-based proteomics of fungal pathogenesis, host–fungal interactions, and antifungal development. J Fungi 5(2), 52. https://doi.org/10.3390/jof5020052

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