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*

NICHOLAS PRUDHOMME

On Tuesday, August 29, 2023 at 1:30 p.m. (SSC 2315)

Thesis Title: **Multi-omics identification of yield limiting factors in molecular pharming**

Examination Committee:

Dr. Priyanka Pundir, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Jennifer Geddes-McAlister, Dept. of Molecular and Cellular Biology
Dr. Emma Allen-Vercoe, Dept. of Molecular and Cellular Biology
Dr. Ian Tetlow, Dept. of Molecular and Cellular Biology
Dr. Glen Uhrig, Dept. of Biological Sciences, University of Alberta (External Examiner)

Advisory Committee:
Dr. Jennifer Geddes-McAlister (Advisor)
Dr. Emma Allen-Vercoe
Dr. Tariq Akhtar

Abstract: Biologics, including protein-based drugs, are among the fastest growing categories of therapeutics under development today. While most biologics are made using mammalian cell systems, plant-based production systems provide a cost-effective approach to making new and similar medicines to fight disease. This process, called molecular pharming, uses a common plant pathogen, *Agrobacterium tumefaciens,* to deliver genes encoding for a specific protein product to the plant host, *Nicotiana benthamiana*. Although plant systems offer excellent potential for therapeutic protein production, barriers, such as plant host defense response, impact yield and limit wider adoption of the technology. In this thesis, I examine the molecular pharming pipeline from a systems-biology perspective to identify putative yield limiting factors. First, I explore the utilization of bioreactors as a substitute for shake flasks in bacterial biomass production. I examine variations in bacterial growth across the tested parameters and establish conditions necessary for consistent bacterial culturing. Through proteomic profiling of cultures under each growth condition, I identify distinct and growth-specific responses in bacterial protein abundance shedding light on the functional roles of these proteins that may influence bacterial processes involved in successful agroinfiltration and transformation. Next, I investigate the impact of time on bacterial culture preparation in agroinfiltration medium. I reveal distinct protein profiles associated with different bacterial treatment conditions and exposure timings, particularly in relation to pathogenesis, motility, and nutrient acquisition systems. These results indicate a progressive cellular remodeling of the bacterium over time to highlight the interconnectedness of pathogenesis and chemotaxis-related proteins with transport and metabolism. Finally, I investigate the infectome of *N. benthamiana* and *A. tumefaciens* to reveal distinctive patterns of bacterial protein production associated with each growth condition, which directly impact plant defense responses and the production of the target protein over time. By combining proteomic and metabolomics profiling, I detect the fluctuation of secondary metabolite production throughout the infection process, providing new insights into the intricate modulation of the dual biological systems. My

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findings suggest that bacterial growth in bioreactors may facilitate the evasion of early plant defense responses against *A. tumefaciens.* Overall, this thesis contributes to the knowledge of molecular pharming and identifies specific factors that may limit the yield of recombinant proteins.

Curriculum Vitae: Nicholas completed his Bachelor of Science in Crop Science with a concentration in Plant Biotechnology and Molecular Biology at the University of Illinois at Urbana-Champaign in May 2015. He began his Master of Science in the lab of Dr. Jennifer Geddes-McAlister in May 2019 and transferred into the PhD in Molecular and Cellular Biology program in September 2020.

Awards: Ontario Graduate Scholarship (2021-2023).

Publications: **Prudhomme N,** Krieger JR, McLean MD, Cossar D, Geddes-McAlister J. Proteomic Profiling of Interplay Between *Agrobacterium tumefaciens* and *Nicotiana benthamiana* for Improved Molecular Pharming Outcomes. *Methods Mol Biol*. 2022;2456:275-286. doi: 10.1007/978-1-0716-2124-0_19. PMID: 35612749.

Geddes-McAlister J, **Prudhomme N**, Gutierrez Gongora D, Cossar D, McLean MD. The emerging role of mass spectrometry-based proteomics in molecular pharming practices. *Curr Opin Chem Biol*. 2022 Jun;68:102133. doi: 10.1016/j.cbpa.2022.102133. Epub 2022 Apr 1. PMID: 35378382.

Prudhomme N, Pastora R, Muselius B, McLean MD, Cossar D, Geddes-McAlister J. Exposure of *Agrobacterium tumefaciens* to agroinfiltration medium demonstrates cellular remodeling and may promote enhanced adaptability for molecular pharming. *Can J Microbiol*. 2021 Jan;67(1):85-97. doi: 10.1139/cjm-2020-0239. Epub 2020 Jul 28. PMID: 32721220.

Prudhomme N, Gianetto-Hill C, Pastora R, Cheung WF, Allen-Vercoe E, McLean MD, Cossar D, Geddes-McAlister J. Quantitative proteomic profiling of shake flask versus bioreactor growth reveals distinct responses of *Agrobacterium tumefaciens* for preparation in molecular pharming. *Can J Microbiol*. 2021 Jan;67(1):75-84. doi: 10.1139/cjm-2020-0238. Epub 2020 Aug 26. PMID: 32846104.

Geddes-McAlister J, Sukumaran A, Patchett A, Hager HA, Dale JCM, Roloson JL, **Prudhomme N**, Bolton K, Muselius B, Powers J, Newman JA. Examining the Impacts of CO_2 Concentration and Genetic Compatibility on Perennial Ryegrass-*Epichloë festucae* var *lolii* Interactions. *J Fungi* (Basel). 2020 Dec 11;6(4):360. doi: 10.3390/jof6040360. PMID: 33322591; PMCID: PMC7770580.

Kristen Van Gelder, Lilia K.A. Virta, Jeremy Easlick, **Nicholas Prudhomme**, Jason A. McAlister, Jennifer Geddes-McAlister, Tariq A. Akhtar. A central role for polyprenol reductase in plant dolichol biosynthesis. *Plant Science*, Volume 303, 2021, 110773, ISSN 0168-9452, https://doi.org/10.1016/j.plantsci.2020.110773.