



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

VICTORIA PETRUCCELLI

on Tuesday, February 16, 2021 at 9:30 a.m. (online)

Thesis Title: Expression of peptidoglycan *O*-Acetyltransferase A from *Neisseria gonorrhoeae*.

Examination Committee:

Dr. John Vessey, Dept. of Molecular and Cellular Biology (Exam Chair)

Dr. Anthony Clarke, Dept. of Molecular and Cellular Biology

Dr. Stephen Seah, Dept. of Molecular and Cellular Biology

Dr. Georgina Cox, Dept. of Molecular and Cellular Biology

Advisory Committee:

Dr. Anthony Clarke (Co-Advisor)

Dr. Chris Whitfield (Co-Advisor)

Abstract: The peptidoglycan (PG) sacculus is a crucial component of most prokaryotic organisms as it prevents cell lysis due to high internal osmotic pressure. The essential control of endogenous lytic transglycosylases (LTs) is provided by the decoration of PG via *O*-acetylation. The existence of *O*-acetyl modifications to the C6 hydroxyl of *N*-acetylmuramic acid residues within the PG of bacteria has been demonstrated to contribute to their pathogenesis by inhibiting the action of lysozymes in the host innate immune system. *O*-Acetylation in Gram-negative bacteria employs a two-component system of an integral membrane protein, peptidoglycan *O*-acetyltransferase (Pat) A, and a periplasmic transferase, PatB. PatA remains uncharacterized but is believed to translocate acetyl groups across the cytoplasmic membrane to subsequently be presented to PatB via an unknown mechanism. This thesis describes studies for the expression of PatA within an *E. coli* expression host, while avoiding toxicity complications. Herein, the apparent expression of PatA from *Proteus mirabilis* was achieved as detected by western immunoblot, but the protein's true identity remains to be confirmed by mass spectrometry. Additionally, biochemical analysis of the periplasm of *N. gonorrhoeae* was used to explore the possibility of a periplasmic molecule to mediate the transfer of acetyl groups from PatA to PatB, but inconsistent results prove that a different approach needs to be taken to assess this hypothesis. These results provide a framework for the expression of PatA so that it may be characterized in future studies, and insight into the possible mechanism for the transfer of acetyl groups.

Curriculum Vitae: Victoria completed her Bachelor of Science (Hons.) at the University of Guelph in the spring of 2018, and then began her MSc in the lab of Dr. Anthony Clarke in the fall of the same year.