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*

LANA EL-OSTA

on Monday, August 27, 2018 at 9:30 a.m. in SSC 2315

Thesis Title: **Biochemical characterization of the putative glucosyltransferase, WapB, which acts on the uncapped core OS of *P. aeruginosa***

Examination Committee:

Dr. R. Lu, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. M. Kimber Dept. of Molecular and Cellular Biology
Dr. S. Graether, Dept. of Molecular and Cellular Biology
Dr. G. Cox, Dept. of Molecular and Cellular Biology

Advisory Committee:
Dr. M. Kimber (Adv)
Dr. J. Lam (Co-Adv.)
Dr. S. Graether

Abstract: *Pseudomonas aeruginosa* is a Gram-negative, opportunistic pathogen known to cause disease in Cystic Fibrosis patients. Lipopolysaccharide (LPS) forms the outer layer of the outer membrane and is an important virulence factor. LPS is organized as with a core oligosaccharide (OS) attached to a glycolipid (lipid A) anchor; in *Pseudomonas*, this core can be synthesized either with or without the addition of an O-antigen. Uncapped (O-antigen free) core is often modified with a terminal glucose (Glc^{IV}) on L-Rha, while a knockout mutant of *wapB* produces LPS lacking this terminal glucose. At present, little is known regarding the enzymatic properties of WapB. My thesis research tests the hypothesis that WapB is a β-1,2-glucosyltransferase which transfers a Glc residue to the uncapped core OS of certain *P. aeruginosa* serotypes. WapB proved to be insoluble when expressed, and many standard solubilization strategies were explored, including: terminal truncations, fusions with solubilisation domains, varying expression hosts, addition of solubilisation agents, and membrane extractions. Of these strategies, membrane extraction using the detergent DDM proved the most promising, suggesting WapB interacts tightly with the membrane. Complementation was also used to show that at least 20 residues at the C-terminus and a DXD motif are required for function.

Curriculum Vitae: Lana obtained her Bachelor of Science (Hons.) at the University of Windsor in the spring of 2016, and then began her M.Sc. in the labs of Dr. Matthew Kimber and Dr. Joseph Lam, in the fall of the same year. For the past two years, Lana was the graduate student representative for the Canadian Society of Microbiology and was part of the MCB committee for Careers in Biology Day.

Publication: Taylor Forrester, Lana El Osta, Joseph S. Lam, Matthew S. Kimber. Polysaccharides: Methods and Protocol. 2018. Chapter: Designing glycosyltransferase expression constructs for improved purification, protein yield and crystallization.