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*

VICTORIA WILSON

On Tuesday, April 12, 2022 at 9:30 a.m. (online)

Thesis Title: **Functional characterization of putative β-lactamases in *Pseudomonas aeruginosa***

Examination Committee:

Dr. John Vessey, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Rebecca Shapiro, Dept. of Molecular and Cellular Biology

Advisory Committee:

Dr. Cezar Khursigara (Advisor)
Dr. Georgina Cox

Abstract: *Pseudomonas aeruginosa* is a Gram-negative, opportunistic pathogen that is a major cause of nosocomial infections in immunocompromised patients. Chronic *P. aeruginosa* infections are difficult to treat due to a limited sensitivity to antimicrobials resulting from an abundance of resistance mechanisms. For example, *P. aeruginosa* has the ability to transition from free roaming (planktonic) cells into biofilms, which provide protection against antimicrobials. *P. aeruginosa* also produces numerous antibiotic-inactivating enzymes such as β-lactamases, which target and break down β-lactam antibiotics. Through proteomic analysis of the *P. aeruginosa* laboratory strain PAO1, a number of uncharacterized proteins were identified that are predicted to be novel β-lactamases. This project focuses on characterizing three of the identified proteins: the biofilm dominant PA2915, the biofilm and planktonic variable PA0832, and the planktonic dominant PA0057. Targeted mutagenesis using allelic exchange was used to remove genes of interest in *P. aeruginosa*. The resulting knockouts were tested for susceptibility to β-lactam antibiotics as both biofilm and planktonic communities. In addition, the target proteins were biochemically characterized *in vitro* by detecting β-lactamase activity using the standard chromogenic β-lactam substrate, nitrocefin. These results show that PA2915 and PA0832 possess low level β-lactamase activity but do not provide any significant resistance against β-lactam antibiotics in *P. aeruginosa*. However, these proteins may play a role in biofilm biomass production in late-stage biofilm formation, as well as during carboxypenicillin stress. PA0057 activity both *in vitro* and *in vivo* was inconclusive. Overall, this work provides the foundation for future structure function studies of these putative β-lactamases.

Curriculum Vitae: Victoria completed her B.Sc (Hons.) in Biochemistry (Co-op) at the University of Guelph in Fall 2019. She began her M.Sc of Molecular and Cellular Biology in the lab of Dr. Khursigara in Winter 2020.

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Awards: Canada Graduate Scholarships – Master's (CGS-M), NSERC (May 2021-2022); American Society for Microbiology Best Poster Award, Canadian Society of Microbiologists (June 2021), awarded for the best poster layout in the 2021 CSM student poster competition.