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*

MARA GOODYEAR

on Thursday, July 14, 2022 at 1:30 p.m. (online)

Thesis Title: **Using a proteomics-based approach to uncover mechanisms of antibiotic resistance in epidemic strains of *Pseudomonas aeruginosa***

Examination Committee:

Dr. Matthew Kimber, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Chris Whitfield, Dept. of Molecular and Cellular Biology
Dr. Matthew Sorbara, Dept. of Molecular and Cellular Biology
Dr. Silvia Cardona, Dept. of Microbiology, University of Manitoba (External Examiner)

Advisory Committee:
Dr. Cezar Khursigara (Advisor)
Dr. Chris Whitfield
Dr. Anthony Clarke

Abstract: *Pseudomonas aeruginosa* is a Gram-negative bacterium that can cause chronic and multidrug-resistant infections. Understanding and predicting the antibiotic resistance profiles of clinical isolates of *P. aeruginosa* is important for developing and administering effective treatments against this opportunistic pathogen. Label-free quantitative proteomics can be used to identify the abundances of proteins produced by clinical isolates of *P. aeruginosa* under different conditions. The proteomics data can then be used to complement genomic, transcriptomic, and phenotypic data to help predict antibiotic resistance profiles, and to identify the molecular mechanisms of antibiotic resistance in clinical isolates of *P. aeruginosa.* In this thesis research, I completed phenotypic and proteomic characterization of isolates of the Liverpool Epidemic Strain (LES) of *P. aeruginosa*. I first provide a comprehensive analysis of the growth and antibiotic susceptibility of eight LES isolates grown as both planktonic and biofilm cultures. Two isolates, LESlike1 and LESB58, with different antibiotic resistance profiles were chosen for proteomic characterization and comparison with the laboratory strain PAO1. In my comparison of the proteomes of LESlike1, LESB58, and PAO1 I demonstrate the potential for proteomic data to predict antibiotic resistance phenotypes that could not be determined from genomic data alone. I next compared the proteomes of LESlike1, LESB58, and PAO1 after they were challenged with one of five treatments (four antibiotics and hydrogen peroxide) to identify their adaptive responses to antibiotic treatment. This analysis identified proteins with different abundances between the three isolates and challenge conditions that can be further characterized to understand their contributions to antibiotic resistance. Specifically, I identified differential abundances of proteins involved in cell wall synthesis and division in LESB58 in

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response to the antibiotic carbenicillin. The inner membrane protein CreD was increased in abundance with high-fold changes in LESB58 and an additional β-lactam resistant isolate exposed to carbenicillin, and I report the results of the initial characterization of knockout strains of *creD*. Overall, this work describes a proteomic dataset that will facilitate further study of protein-based resistance in clinically important isolates of *P. aeruginosa.*

Curriculum Vitae: Mara completed her Bachelor of Science (Hons) in Microbiology at the University of Guelph in Winter 2015. She then began her Doctor of Philosophy in Molecular and Cellular Biology in Fall 2015 under the supervision of Dr. Cezar Khursigara.

Awards: NSERC Canada Graduate Scholarship – Doctoral (2016-2018); Career and Teaching Development Fellowship, University of Guelph (2019); Pharmacia MCB Graduate Prize for Best Poster (2017); NSERC Canada Graduate Scholarship – Masters (2015)

Publications: **Goodyear MC**, Garnier NE, Levesque, RC, Khursigara, CM. (2022). Liverpool Epidemic Strain isolates of *Pseudomonas aeruginosa* display high levels of antimicrobial resistance during both planktonic and biofilm growth. *Microbiology Spectrum*. e01024-22.

Gheorghita AA, Wolfram F, Whitfield GB, Jacobs HM, Pfoh R, Wong SSY, Guitor AK, **Goodyear MC**, Berezuk AM, Khursigara CM, Parsek MR, Howell PL. (2022). The *Pseudomonas aeruginosa* homeostasis enzyme AlgL clears the periplasmic space of accumulated alginate during polymer biosynthesis. *Journal of Biological Chemistry*. 298(2):101560.

Goodyear MC, Garnier N, Krieger JR, Geddes-McAlister J, Khursigara CM. (2021). Label-free quantitative proteomics identifies unique proteomes of clinical isolates of the Liverpool Epidemic Strain of *Pseudomonas aeruginosa* and laboratory strain PAO1. *Proteomics – Clinical Applications.* 15(6): e2100062.

Berezuk AM, Glavota, S, Roach EJ, **Goodyear MC**, Krieger JR, Khursigara CM. (2018). Outer membrane lipoprotein RlpA is a novel periplasmic interaction partner of the cell division protein FtsK in *Escherichia coli*. *Scientific Reports.* 8:12933.

Habash MB, **Goodyear MC**, Park AJ, Surette MD, Vis EC, Harris RJ, Khursigara CM. (2017). Potentiation of tobramycin by silver nanoparticles against *Pseudomonas aeruginosa* biofilms. *Antimicrobial Agents and Chemotherapy.* 61(11): e00415-17.

Berezuk AM, **Goodyear M**, Khursigara CM. (2014). Site-directed fluorescence labeling reveals a revised N-terminal membrane topology and functional periplasmic residues in the *Escherichia coli* cell division protein FtsK. *Journal of Biological Chemistry.* 289(34): 23287-23301.

Toh MC, **Goodyear M**, Daigneault M, Allen-Vercoe E, Van Raay TJ. (2013). Colonizing the embryonic zebrafish gut with anaerobic bacteria derived from the human gastrointestinal tract. *Zebrafish.* 10(2): 194-198.