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*

LAURA THOMPSON

on Friday, January 17th, 2024 at 1:00p.m. (SSC 2315)

Thesis Title: Genomic Tools to Investigate Bacterial Drug Efflux Pumps

Examination Committee:

Dr. Steffen Graether, Molecular and Cellular Biology (Exam Chair)
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Jennifer Geddes-McAlister, Dept. of Molecular and Cellular Biology
Dr. Cullen Myers, Dept. of Molecular and Cellular Biology
Dr. Jean-Philippe Côté, biology Department, University of Sherbrooke (External Examiner)

Advisory Committee:
Dr. Georgina Cox (Adv)
Dr. Chris Whitfield
Dr. Cezar Khursigara
Dr. Jennifer Geddes-McAlister

Abstract: New therapeutics are urgently needed to combat Gram-negative pathogens such as *Escherichia coli*, yet antibiotic development has been significantly hindered due to intrinsic antimicrobial resistance elements such as efflux pumps. *E. coli* harbours 35 known or putative efflux pumps, many of which confer antimicrobial resistance. However, the physicochemical properties and molecular descriptors that govern efflux are poorly understood due to the complexity of the efflux network and the lack of suitable genetic background. The overarching goal of this thesis was to develop genomic tools to better understand efflux pump activity. First, the conservation of efflux-encoding genes in the *E. coli* core genome was assessed to provide insight into the evolution and phylogenetic relationships of these proteins. Next, an efflux deficient mutant devoid of 35 efflux-encoding genes was generated, Efflux KnockOut-35 (EKO-$35_{2.0}$), forming the basis of a genetic platform to introduce isolated efflux pumps and delineate their activity. The *E. coli* Efflux Platform was profiled against a curated and diverse compound collection to characterize the substrate profiles of drug efflux pumps and determine the physicochemical properties that govern compound transport in *E. coli*. A machine learning model was developed to define trends in the efflux-susceptible chemical space, identifying key molecular descriptors that differentiate efflux-susceptible and avoidant molecules. Lastly, the *E. coli* Efflux Platform was used to investigate novel physiological functions of efflux pumps, identifying efflux pump-encoding genes that contribute to acidic pH homeostasis. Overall, this research provides invaluable genomic and bioinformatic tools to enable the development of efflux-avoidant antimicrobials, enhances fundamental knowledge of these resistance elements, and guides efforts to overcome intrinsic resistance.

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Curriculum Vitae: Laura obtained her Bachelor of Science (Honours, Co-op) at the University of Guelph in 2019. In the winter of 2020, she entered the MSc. program under the supervision of Dr. Georgina Cox. In the winter of 2021, she transferred into the Ph.D. program.

Awards: NSERC Postgraduate Canadian Scholarship – Doctoral (2021-2024)
NSERC Canadian Graduate Scholarship - Masters (2020-2021)
Ontario Graduate Scholarship - Masters (2020)
Ontario Graduate Scholarship - Doctoral (2024-2025)
Donald R. Phillips Molecular and Cellular Biology Scholarship (2024)
CIHR Travel Award (2024)
Braithwaite Conference Travel Grant (2024)
Canadian Society of Microbiology Burrows Award for Womxn in Microbiology (2022)
Arthur Richmond Memorial Scholarship (2022)
Canadian Society of Microbiology Cedarlane Student Symposium Award (2021)
Roche Molecular Biochemicals Award of Excellence (2021)

Publications: Teelucksingh[1] T., **Thompson[1] L.K.**, Cox G. (2020) The evolutionary conservation of *Escherichia coli* drug efflux pumps supports physiological functions. *J. Bacteriol.* 202: e00367-20. **1 – co-first authors**

Teelucksingh[1] T., **Thompson[1] L.K.**, Zhu S., Kuehfuss N.M., Goetz J.A., Gilbert S.E., MacNair C.R., Geddes-McAlister J., Brown E.D., Cox G. (2022). A genetic platform to investigate the functions of bacterial drug efflux pumps. *Nat. Chem. Biol.* 18: 1399-1409. **1 – co-first authors**

Goetz J.A., Kuehfuss N.M, Botschner A.J., Zhu S., **Thompson L.K.,** Cox G. (2022) Exploring functional interplay amongst *Escherichia coli* efflux pumps. *Microbiol.* 168: 001261.

Leonard A.C., Goncheva M.I., Gilbert S.E., Shareefdeen H., Petrie L.E., **Thompson L.K.**, Khursigara C.M., Heinrichs D.E., Cox G. (2023). Autolysin-mediated peptidoglycan hydrolysis is required for the surface display of *Staphylococcus aureus* cell wall-anchored proteins. *Proc Nat Acad Sci.* 120(12), e2301414120.

Wright M., Kaur M., **Thompson L.K.,** Cox G. (2024). A historical perspective on TolC: The multifunctional outer membrane channel protein in *Escherichia coli*. *Npj Antimicrob. Resist.* (In press).

Patent Applications: Cox, G. Teelucksingh, T., **Thompson, L.K.** Genetic platform to investigate the functions of bacterial drug efflux pumps. United States Patent US20230407243A1. Application number 18/210, 416. December 21, 2023.