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*

CAITLIN SANDE

On Tuesday, February 14, 2023 at 1:30 p.m. (SSC 1511)

Thesis Title: **Investigating the genetic determinants of *Escherichia coli* capsular polysaccharide synthesis**

Examination Committee:

Dr. Michael Emes, 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. Matthew Sorbara, Dept. of Molecular and Cellular Biology
Dr. Matthew Jorgenson, Dept. of Microbiology and Immunology, University of Arkansas for Medical Sciences (External Examiner)

Advisory Committee:
Dr. Chris Whitfield (Advisor)
Dr. Cezar Khursigara
Dr. Georgina Cox
Dr. Eric Brown

Abstract: Bacterial capsules are protective layers of high molecular weight polysaccharides that surround the cell surfaces of many Gram-negative bacterial pathogens. Capsular polysaccharides (CPSs) show remarkable structural diversity; in *Escherichia coli* alone, there are approximately 80 K- (capsular) antigen serotypes. In many pathogens, CPSs are often essential for cell survival and virulence within the host because they allow evasion of immune defenses. The documented attenuation of unencapsulated mutants in animal models of infection makes CPS biosynthesis and export proteins candidate therapeutic targets. However, such "antivirulence" applications require a fundamental understanding of the underpinning cellular processes.

Despite their structural diversity, CPSs are formed by one of two conserved assembly strategies. The *E. coli* "group 2" capsule systems are defined by an assembly strategy with polymer export dependent on an ATP-binding cassette (ABC) transporter. The dedicated proteins involved in the synthesis and export of *E. coli* group 2 CPSs are known. In contrast, other supporting cellular components involved in CPS expression, and the global connectivity of this pathway with the other cellular processes, have not been established. Here, I used genome-wide phenotypic screening as an unbiased approach to identify "housekeeping" genes necessary for capsule assembly. Surprisingly, the list of newly discovered genes required for group 2 CPS biosynthesis is brief and is often dependent on growth conditions, indicating *kps* functionality is mostly independent of genetic background, and yet is highly responsive to environmental cues.

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The conserved requirements of housekeeping elements across CPS assembly strategies have yet to be well studied. The requirement for Braun's lipoprotein (Lpp; a key protein in the maintenance of outer membrane integrity and essential in the assembly of the *E. coli* group 2 CPS export machinery) in capsule assembly by different strategies is investigated in this thesis. The results show that the importance of Lpp varies depending on the structure of the outer membrane CPS translocon.
These studies offer important new insight into CPS assembly in Gram-negative bacteria and the relationship between the CPS biosynthesis pathway and other cellular processes.

Curriculum Vitae: Caitlin completed her BSc Honours in Biochemistry, Co-op at the University of Guelph in December 2016. She then began her PhD in Molecular and Cellular Biology under the supervision of Dr. Chris Whitfield in January 2017.

Awards: Canadian Graduate Scholarship – Doctoral, Canadian Institutes of Health Research (2019); Ontario Graduate Scholarship (2018); Canadian Graduate Scholarship – Masters, Natural Sciences and Engineering Research Council of Canada (2017); University of Guelph Graduate Excellence Entrance Scholarship (2017).