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*

LIAM DOYLE

on Friday, April 30, 2021 at 1:30 p.m. (online)

Thesis Title: **Assembly of conserved glycolipid termini in Gram-negative bacterial capsules synthesized by ABC transporter-dependent pathways.**

Examination Committee:

Dr. Ray Lu, Molecular and Cellular Biology (Exam Chair)
Dr. Anthony Clarke, Molecular and Cellular Biology
Dr. Dr. Matthew Kimber, Molecular and Cellular Biology
Dr. Dr. Stephen Seah, Molecular and Cellular Biology
Dr. David Vocadlo, Chemistry, Simon Fraser University (External Examiner)

Advisory Committee:

Dr. Chris Whitfield (Adv)
Dr. Matthew Kimber
Dr. Anthony Clarke

Abstract: The cell surfaces of Gram-negative bacteria are densely populated by complex sugar-containing macromolecules, known as glycoconjugates. These glycoconjugates provide survival advantages, including protection from the mammalian immune system. Capsular polysaccharides (CPSs) represent a prevalent class of serotype-specific surface glycoconjugates which are well-established virulence factors. Despite the incredible structural diversity of CPS molecules, their assembly strategies are limited. In one major assembly pathway in Gram-negative bacteria, the CPS glycan is polymerized in the cytoplasm on a phospholipid acceptor and is exported across the cytoplasmic membrane using an ATP-binding cassette (ABC) transporter. Two retaining 3-deoxy-β-d-*manno*-oct-2-ulosonic acid (β-Kdo) glycosyltransferase (GT) enzymes, KpsS and KpsC (in *Escherichia coli* nomenclature), have been implicated in initiating CPS biosynthesis and are essential to CPS production in this system. This work provides the biosynthetic pathway of *E. coli* K1 CPS through the biochemical characterization of the KpsS and KpsC GTs. These findings offer a model for initiation of CPS biosynthesis across the range of Gram-negative bacteria, which possess KpsS and KpsC homologs and use an ABC transporter-dependent assembly pathway. The crystal structures, determined here for the individual KpsC GTs, share global architectural similarity and are the founding members of the GT107 enzyme family. Their structures resemble GT-B enzymes, composed of two Rossmann-like α/β domains. However, these domains in KpsC GTs are reduced in size

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compared to canonical GT-B enzymes, particularly the N-terminal α/β domain responsible for acceptor binding, and the presence of a helical sub-domain is a feature not found in enzymes outside of the β-Kdo GTs. In summary, this thesis provides insight into the conserved enzymes involved in ABC transporter-dependent CPS production which are found in clinically-relevant Gram-negative bacteria.

Curriculum Vitae: Liam began his graduate program in the lab of Dr. Chris Whitfield, immediately following completion of his Bachelor of Science (Honours) degree at the University of Guelph in April 2016. While he initially started in the M.Sc. program, Liam later transferred directly to the Ph.D. program the following year.

Awards: University of Guelph Dr. Donald R. Phillips Scholarship (2020)
NSERC Postgraduate Scholarship - PGS-D (2018)
NSERC Canada Graduate Scholarship - CGS-M (2017)

Publications: **Doyle, L.**, Ovchinnikova, O.G., Myler, K., Mallette, E., Huang, B.S., Lowary, T.L., Kimber, M.S., and Whitfield, C. (2019). Biosynthesis of a conserved glycolipid anchor for Gram-negative bacterial capsules. Nat. Chem. Biol. *15*, 632–640.

Ovchinnikova, O.G., **Doyle, L.**, Huang, B.-S., Kimber, M.S., Lowary, T.L., and Whitfield, C. (2016). Biochemical characterization of bifunctional 3-deoxy-β-D-*manno*-oct-2-ulosonic acid (β-Kdo) transferase KpsC from *Escherichia coli* involved in capsule biosynthesis. J. Biol. Chem. *291*, 21519–21530.