



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

SEAN LISTON

on Monday, April 30, 2018 at 1:30 p.m. in SSC 2315

Thesis Title: Biosynthesis and assembly of the Vi antigen capsule produced by *Salmonella enterica* serovar Typhi

Examination Committee:

Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Exam Chair)

Dr. C. Whitfield, Dept. of Molecular and Cellular Biology

Dr. J. Wood, Dept. of Molecular and Cellular Biology

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Dr. T. Moraes, Dept. of Biochemistry, University of Toronto

Advisory Committee:

Dr. C. Whitfield (Adv)

Dr. J. Lam

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Abstract: Capsules are bacterial cell-surface structures composed of hydrated capsular polysaccharides (CPS). The human pathogen *Salmonella enterica* serovar Typhi produces the 'Vi antigen' CPS, which contributes to virulence and is used as a vaccine. Vi antigen capsule assembly employs an ATP-binding cassette (ABC) transporter, an export strategy that is conserved in diverse encapsulated bacteria. CPS ABC transporters participate in heterologous protein complexes that are proposed to form enclosed conduits to the cell surface. This thesis describes the identification of Vi antigen biosynthesis genetic loci in the *Burkholderiales* that paradoxically encode a predicted polysaccharide de-polymerase, that I named VexL. Biochemical analyses of *Achromobacter denitrificans* VexL demonstrated that it is a Vi antigen-specific endo-lyase. Determinants of substrate specificity were revealed by a 1.2-Å crystal structure of the VexL-Vi antigen complex. When introduced into *S. Typhi*, VexL localized to the periplasm and degraded nascent Vi antigen, whereas a cytosolic derivative had no effect unless export was disrupted. This provides the first evidence that a CPS is periplasm-exposed during envelope translocation.

Vi antigen-producers lack enzymes to build a terminal glycolipid that is conserved in all other CPS assembled using an ABC transporter. VexL was used to depolymerize Vi antigen, and then intact glycan termini were isolated. Mass spectrometry revealed a di-β-hydroxyacyl-N-acetylhexosamine residue linked to the reducing terminus of Vi antigen. The VexE protein is uniquely encoded in Vi antigen biosynthesis loci and is homologous to acyltransferases from lipid A biosynthesis. A Δ*vexE*

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mutant of *S. Typhi* produced Vi antigen with altered physical properties; its export was impaired, secreted glycan was not attached at the cell surface, and the glycolipid was not identified. Biochemical assays demonstrate that VexE is a UDP-*N*-acetylglucosamine C6 β -hydroxyacyltransferase that prefers 14-carbon acyl chains. The structure of the terminal glycolipid dictates a unique assembly mechanism and has potential implications in pathogenesis and vaccine production. This thesis provides insight into mechanisms for CPS production conserved in diverse bacterial pathogens.

Curriculum Vitae: Sean obtained his Bachelor of Science (Honours) at the University of Guelph in 2013. In the Fall of 2013, he entered directly in to the Ph.D. program with Dr. Chris Whitfield as his advisor.

Awards: Dr. Donald R. Phillips Molecular and Cellular Biology Graduate Scholarship (2016); NSERC CGS – Michael Smith Foreign Study Supplement (2016); Arthur Richmond Memorial Scholarship (2015); NSERC Canada Graduate Scholarship-Doctoral (2015-2018); U of G Dean’s Tri-Council Scholarship (2015-2018); Ontario Graduate Scholarship (2015); Roche Molecular Biochemicals Award of Excellence (2014); Ontario Graduate Scholarship (2014); MCB Summer Research Award (2012).

Publications: Liston, S. D., McMahon, S. A., LeBas, A., Suits, M.D.L., Naismith, J.H., and Whitfield, C. (2018). A periplasmic depolymerase provides new insight into ABC transporter-dependent secretion of bacterial capsular polysaccharides. *Proc. Natl. Acad. Sci. U. S. A.* In Press.

Liston, S. D., Mann, E., and Whitfield, C. (2017). Glycolipid substrates for ABC transporters required for the assembly of bacterial cell-envelope and cell-surface glycoconjugates. *BBA-Mol Cell Biol L.* **1862(11)**, 1394-1403.

Liston, S. D., Ovchinnikova, O. G., Whitfield, C. (2016). Unique lipid anchor attaches Vi antigen capsule to the surface of *Salmonella enterica* serovar Typhi. *Proc. Natl. Acad. Sci. U. S. A.* **113(24)**, 6719-6724.

Liston, S. D., Clarke, B. R., Greenfield, L. K., Richards, M. R., Lowary, T. L., and Whitfield, C. (2015). Domain interactions control complex formation and enzyme specificity in the biosynthesis of the *Escherichia coli* O9a antigen. *J. Biol. Chem.* **290(2)**, 1075-1085.