



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

SAMANTHA WEAR

on Tuesday, April 19, 2022 at 9:30 a.m. (online)

Thesis Title: Investigation of two polysaccharide ‘polymerases’ involved in the synthesis of bacterial cell-surface glycans

Examination Committee:

Dr. Ray Lu, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Matthew Sorbara, Dept. of Molecular and Cellular Biology
Dr. Miguel Valvano, School of Medicine, Dentistry and Biomedical Sciences,
Queen’s University of Belfast (External Examiner)

Advisory Committee:

Dr. Chris Whitfield (Adv)
Dr. Georgina Cox
Dr. Cezar Khursigara

Abstract: Cell-surface polysaccharides serve as essential virulence determinants for many bacteria. These molecules are structurally diverse, as are the activities of the glycosyltransferase (GT) enzymes involved in their synthesis. Most GTs catalyze a single transfer/linkage, whereas those which transfer multiple sugars are referred to as processive GTs or polymerases and are the focus of the research herein. Some polymerases, often referred to as synthases, have the additional capacity to both polymerize and export glycans across the membrane. One example is the O:54 antigen synthase, WbbF, from *Salmonella enterica* serovar Borreze. WbbF differs from prototypical synthases in the requirement for a lipid carrier. I examined the membrane topology of WbbF and used site-directed mutagenesis to confirm that residues important for catalysis and processivity in known synthases are conserved in WbbF. These findings indicate that the GT mechanism of WbbF and classic synthases are likely conserved, despite the use of a lipid acceptor.

Regardless of the biological and biotechnological importance of the virulence capsular polysaccharide (Vi antigen) of *S. enterica* serovar Typhi, some central aspects of its production are poorly understood. The production of Vi antigen was hypothesized to employ a single polymerase. Here I demonstrate that TviE and TviD are the Vi antigen polymerase and *O*-acetyltransferase, respectively. Structural modeling and properties of site-directed mutants reveal TviE to be a GT4 enzyme. TviD contains an N-terminal *O*-acetyltransferase, verified by the structure of the product made by a *tviD*-mutant polysaccharide. Site-directed mutagenesis determined that TviD possesses an atypical catalytic triad compared to members of the SGNH hydrolase family. Tetratricopeptide repeats at the C-terminus of TviD mediate interactions

between TviD and TviE. Vi antigen is also produced by environmental *Bordetella* isolates, while mammal-adapted *Bordetella* species (like *B. bronchiseptica*) produce Vi antigen-related capsule of undetermined structure that cross-reacts with antibodies recognizing Vi antigen. The *B. bronchiseptica* genetic locus predicts a different mode of synthesis from classical Vi antigen but still involves TviD and TviE homologs that are both active in a reconstituted *S. Typhi* system. This research provides foundational information for biosynthesis of important classes of polysaccharides and the structures and activities of polymerases with different properties.

Curriculum Vitae: Samantha completed her Bachelor of Science in Biochemistry at the University of Guelph in the Spring of 2015. She began her graduate studies in the lab of Dr. Whitfield in the Fall of 2015 in the MSc program. Samantha later transferred to the PhD program in the Winter of 2017.

Publications: **Wear, S.S.**, Sande, C., Ovchinnikova, O.G., Preston, A., and Whitfield, C. (2021). Investigation of core machinery for biosynthesis of Vi antigen capsular polysaccharides in Gram-negative bacteria. *J. Biol. Chem.* 298, 101486.

Whitfield, C., **Wear, S.S.**, and Sande, C. (2020). Assembly of bacterial capsular polysaccharides and exopolysaccharides. *Annu. Rev. Microbiol.* 74, 521-543.

Wear, S.S., Hunt, B.A., Clarke, B.R., and Whitfield, C. (2020). Analysis of the topology and active-site residues of WbbF, a putative O-polysaccharide synthase from *Salmonella enterica* serovar Borreze. *J. Bacteriol.* 202, e00625-19.

Sande, C., **Wear, S.**, and Whitfield, C. (2019). Capsules and secreted extracellular polysaccharides. In *Encyclopedia of Microbiology*, T.M. Schmidt, ed. (Cambridge, MA: Academic Press), pp. 604-612.