



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

TAYLOR FORRESTER

On Friday, August 4, 2023 at 9:30 a.m. (SSC 2315)

Thesis Title: Investigating diversity in O antigen polysaccharide glycosyltransferases: structures, mechanisms, and regulation

Examination Committee:

Dr. Michael Emes, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Chris Whitfield, Dept. of Molecular and Cellular Biology
Dr. Siavash Vahidi, Dept. of Molecular and Cellular Biology
Dr. Marcelo Guerin, BioCruces Bizkaia Health Research Institute (External Examiner)

Advisory Committee:

Dr. Matthew Kimber (Advisor)
Dr. Georgina Cox
Dr. Stephen Seah
Dr. Chris Whitfield

Abstract: O antigen polysaccharides (OPSs) are repetitive sugar polymers that decorate the cell surface of Gram-negative bacteria. OPSs facilitate interactions with potential hosts (both pathogenically and symbiotically), protect against bacteriophage predators and form a barrier to harmful small molecules. These interactions are dictated by the specific composition and length of the OPS, which show extreme variation between and even within species. A large class of enzymes, known as glycosyltransferases (GTs), is responsible for polymerizing the OPS from individual monosaccharides, linked together in a regio- and stereo-specific manner, often to discrete lengths. The research in this thesis explores several aspects of O antigen biosynthesis, with a focus on diverse examples of GTs which produce them. First, using the β -Kdo GT Wbb_{GT99} from *Raoultella terrigena*, I thoroughly detail the first unambiguous use of a double displacement mechanism in GTs through a combination of structural and biochemical approaches. Second, a novel type IIb system for OPS chain-length regulation is proposed for *Rhizobium tropici* CIAT 899, based on biochemical analysis of the functional enzymes present in the cluster. This strategy substitutes a β -helix spacer domain for α -helical coiled-coils in key enzyme, ORF3. Finally, the fucosyltransferase, ORF6, from the *R. tropici* OPS cluster was the subject of a thorough structure-function analysis which revealed a new metal-independent GT family. Overall, this thesis describes the catalytic mechanisms for two divergent GTs in detail, advancing our understanding of GT mechanisms, and highlights the initial characterization of the *R. tropici* OPS biosynthesis cluster, providing evidence for a novel strategy of glycan length control.

Curriculum Vitae: Taylor completed his Bachelor of Science (Honours, Co-op) in Biochemistry at the University of Guelph in Fall 2016. He started a direct-entry PhD in Molecular and Cellular Biology in Fall 2017 under the supervision of Dr. Matthew Kimber.

Awards: Donald R. Philips Molecular and Cellular Biology Scholarship (2023); Queen Elizabeth II Graduate Scholarship in Science and Technology (2020 – 2021); Ontario Graduate Scholarship (2019 – 2020); Graduate Excellence Entrance Scholarship (2017)

Publications: Doyle, L., Ovchinnikova, O.G., Huang, B., **Forrester, T.J.B.**, Lowary, T.L., Kimber, M.S., and Whitfield, C.W. (2023) Investigation of the mechanism and linkage specificities of the retaining β -Kdo glycosyltransferase modules of KpsC. *The Journal of Biological Chemistry*. 299(5): 104609

Forrester, T. J. B., Ovchinnikova, O. G., Li, Z., Kitova, E. N., Nothof, J. T., Koizumi, A., Klassen, J. S., Lowary, T. L., Whitfield, C., and Kimber, M. S. (2022) The retaining β -Kdo glycosyltransferase WbbB uses a double-displacement mechanism with an intermediate adduct rearrangement step. *Nature Communications*. 13: 6277

Boddington, K.F., Soubeyrand, E., Van Gelder, K., Casaretto, J.A., Perrin, C., **Forrester, T.J.B.**, Parry, C., Al-Abdul-Wahid, M.S., Jentsch, N.G., Magolan, J., Bozzo, G.G., Kimber, M.S., Rothstein, S.J., Akhtar, T.A. (2021). Bibenzyl synthesis in *Cannabis sativa* L. *The Plant Journal*. 109(3): 693-707

Forrester, T.J.B., El Osta, L., Lam, J. S., and Kimber, M. S. (2019). Chapter 11: Designing glycosyltransferase expression constructs for improved purification, protein yield and crystallization. *Bacterial Polysaccharides: Methods and Protocols*. 1954: 137-150

Ryan, P., **Forrester, T.J.B.**, Wroblewski, C., Kenney, T.M.G., Kitova, E. N., Klassen, J. S., and Kimber, M.S. (2019). The small RbcS-like domains of the β -carboxysome structural protein, CcmM, bind RubisCO at a site distinct from that binding the RbcS subunit. *The Journal of Biological Chemistry*. 294(8): 2593-2603