



Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of **Doctor of Philosophy** of

STEVEN HUSZCZYNSKI

on Tuesday, December 18, 2018 at 1:30 p.m. in SSC 2315

Thesis Title: Genetic and biochemical characterization of the proteins that determine O-specific antigen chain length in *Pseudomonas aeruginosa*

Examination Committee:

Dr. R. Lu, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. C. Khursigara, Dept. of Molecular and Cellular Biology
Dr. S. Seah, Dept. of Molecular and Cellular Biology
Dr. L. Mutharia, Dept. of Molecular and Cellular Biology
Dr. K. Maxwell, University of Toronto, MaRs Centre

Advisory Committee:

Dr. J. Lam (Adv.)
Dr. C. Khursigara (Co-Adv.)
Dr. S. Seah
Dr. R. Merrill

Abstract: The surfaces of bacteria are often decorated with complex polysaccharides that function as protective barriers and mediate interactions with the extracellular environment. One such molecule is lipopolysaccharide (LPS), the main constituent of the extracellular face of a prototypical Gram-negative bacterial outer membrane. LPS is a major virulence factor composed of a lipid anchor, core oligosaccharide, and a long polysaccharide chain, termed the O antigen, which is highly variable in structure and length. Importantly, the regulation of the O antigen chain to predetermined lengths may confer specific protective properties and/or facilitate certain interactions. This work examines the assembly of the O-specific antigen (OSA) glycoform of O antigen in the opportunistic pathogen *Pseudomonas aeruginosa* and investigates the mechanisms that determine the length of the polymer chain. In order to broaden our understanding of OSA biosynthesis we aimed to locate the previously unidentified O antigen biosynthesis clusters responsible for the synthesis of the O15 and O17 OSA structures by mining published whole genome sequence data. Both clusters were found outside of the conserved OSA biosynthesis locus and were likely acquired through multiple horizontal gene transfer events. The results of our knockout and overexpression experiments determined that synthesis of the O15 and O17 antigens follows an ABC transporter-dependent pathway previously unidentified in *P. aeruginosa*. We also discovered that the O15 and O17 polysaccharide chain lengths are regulated by “molecular rulers” with distinct domain architectures. In a separate investigation, we examined OSA chain length regulation in the Wzx/Wzy-dependent pathway. In this system, two Wzz proteins, Wzz₁ and Wzz₂, confer OSA chain lengths of long and very long, respectively. We found that compared to Wzz₁, Wzz₂ has distinct amino acid insertions in the central α -helices and in membrane distal and proximal loops. When these regions

were deleted in Wzz₂, the mutant proteins conferred drastically shortened chain lengths. Within these regions we identified several conserved amino acid residues that were then targeted for site-directed mutagenesis. Our results implicate an RXE motif in loop 4 and a “hot spot” of charged and polar residues in $\alpha 7$ in the function of Wzz₂. We present evidence that the functionally important residues of $\alpha 7$ are likely involved in stabilizing Wzz₂ through coiled-coil interactions. Overall, the results discussed here provide a comprehensive look at *P. aeruginosa* O antigen assembly by two distinct pathways, which will be applicable to the study of a range of important bacterial glycoconjugates.

Curriculum Vitae: Steven obtained his Bachelor of Science (Honours) at the University of Guelph in 2013. He then started as a Master’s student in the laboratory of Dr. Joe Lam, and transferred to the PhD program in 2015. Steven’s co-advisor is Dr. Cezar Khursigara.

Awards: Ontario Graduate Scholarship (2013, 2015, 2016)

Canadian Institute of Health Research Canada Graduate Scholarship – Masters (2014)

Publications: Taylor, V. L., Hoage, J. F., Thrane, S. W., **Huszczynski, S. M.**, Jelsbak, L., & Lam, J. S. (2016). A Bacteriophage-Acquired O-Antigen Polymerase (Wzy β) from *P. aeruginosa* Serotype O16 Performs a Varied Mechanism Compared to Its Cognate Wzy α . *Frontiers in microbiology*, 7, 393. doi:10.3389/fmicb.2016.00393

Taylor V.L., **Huszczynski S.M.**, Lam J.S. (2015) Membrane Translocation and Assembly of Sugar Polymer Precursors. In: Bagnoli F., Rappuoli R. (eds) Protein and Sugar Export and Assembly in Gram-positive Bacteria. Current Topics in Microbiology and Immunology, vol 404. Springer, Cham

Islam, S. T., **Huszczynski, S. M.**, Nugent, T., Gold, A. C., & Lam, J. S. (2013). Conserved-residue mutations in Wzy affect O-antigen polymerization and Wzz-mediated chain-length regulation in *Pseudomonas aeruginosa* PAO1. *Scientific reports*, 3, 3441. doi:10.1038/srep03441

Hao, Y., King, J. D., **Huszczynski, S.**, Kocíncová, D., & Lam, J. S. (2013). Five new genes are important for common polysaccharide antigen biosynthesis in *Pseudomonas aeruginosa*. *mBio*, 4(1), e00631-12. doi:10.1128/mBio.00631-12