COLLEGE OF BIOLOGICAL SCIENCE
Department of Molecular and Cellular Biology

ANNOUNCEMENT: Interested members of the University Community are invited to attend the Final Oral Examination for the Degree of **Doctor of Philosophy** of

Francesca A. N. Herlihey

of the Department of Molecular and Cellular Biology
on Tuesday, January 24, 2017 at 9:30 a.m. in SSC 1511

Thesis Title: **Biochemical Characterization of the Activity and Control of the Autolysins Involved in Flagella Assembly in Gram-Negative Bacteria**

Examination Committee:
Dr. M. Brauer, Dept. of Molecular and Cellular Biology (Chair)
Dr. A. Clarke, Dept. of Molecular and Cellular Biology
Dr. D. Brewer, Mass Spectrometry Lab, Advanced Analysis Centre
Dr. C. Khursigara, Dept. of Molecular and Cellular Biology
Dr. B. Mark, Dept. of Microbiology, University of Manitoba

ABSTRACT

Francesca Herlihey B.Sc. (Hons.) **Advisor: Dr. Anthony Clarke**

An important step during flagellum assembly is the localized and controlled degradation of the peptidoglycan sacculus to allow for the insertion of the rod as well as to facilitate anchoring for proper motor function. The lysis events necessary for the insertion of cellular machinery as well as cellular growth and division require enzymes such as lytic transglycosylases and β-N-acetylglucosaminidases. Due to their structural similarities and use of crude assays, the nature of the lytic activity of many β- glycosidases remains unknown. I present the development of a novel assay for glycoside lytic enzymes and its use to provide the enzymatic characterization of the lytic domain of the dedicated flagellar enzymes FlgJ and SltF from *Salmonella* Typhimurium and *Rhodobacter sphaeroides*, respectively. In β- and γ-proteobacteria, FlgJ functions as a β-N-acetylglucosaminidase while in α-proteobacteria SltF functions as a lytic transglycosylase. Given their lethal potential, these enzymes are regulated at the enzyme level in many bacteria. The control of SltF's activity was investigated and it is modulated by two flagellar rod proteins, FlgB and FlgF; FlgB stabilizes and stimulates SltF activity while FlgF inhibits it. In addition, the specificity of the Ivy proteins, inhibitors originally identified as inhibitors of vertebrate lysozyme, was investigated. Ivyp1 and Ivyp2 from *Pseudomonas aeruginosa* were found to be more specific for the soluble lytic transglycosylases SltB1 and Slt70 than for lysozymes. Further insight into the modulation of the sacculus may provide new avenues for the development of novel antibacterials.

CURRICULUM VITAE:

Francesca completed her BSc. Biochemistry (Honours), with a minor in Mathematical Sciences, at the University of Guelph in April 2011. She began her graduate studies in Dr. Clarke's lab in September 2011.

PUBLICATIONS:

Herlihey, F. A., Osorio-Valeriano, M., Dreyfus, G., and Clarke, A. J. (2016) Modulation of the lytic activity of the dedicated autolysin for flagellum formation SltF by flagellar rod proteins FlgB and FlgF. *J Bacteriol* 198, 1847-1856 - This article was highlighted in the July 2016 issue of the journal as an article of significant interest selected by the editors.

Herlihey, F. A., Moynihan, P. J., and Clarke, A. J. (2014) The essential protein for bacterial flagella formation FlgJ functions as a β-*N*-acetylglucosaminidase. *J Biol Chem* 289, 31029-31042

Herlihey, F. A., and Clarke, A. J. (2016) Controlling autolysis during flagella insertion in Gram- negative bacteria. *Protein Reviews*, Springer US, Boston, MA. pp 1-16