UNIVERSITY of GUELPH

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 **Master of Science** of

JOSEPH CIUFO, on Thursday, November 30, 2017 at 9:30 a.m. in SSC 2315
(Advisor: Dr. A. Clarke)

Thesis Title: **Inhibition of membrane-bound lytic transglycosylases A, B and F by membrane-bound lysozyme inhibitor of C-type lysozyme.**

Examination Committee:

Dr. J. Vessey, Dept. of Molecular and Cellular Biology (Chair)
Dr. A. Clarke, Dept. of Molecular and Cellular Biology
Dr. C. Khursigara, Dept. of Molecular and Cellular Biology
Dr. L. Mutharia, Dept. of Molecular and Cellular Biology

Abstract: Peptidoglycan (PG) is an essential component of bacterial cells that forms a mesh-like sacculus that surrounds the cell. Despite its structural role, PG is constantly being remodeled by an endogenous class of enzymes known as lytic transglycosylases (LTs). LTs are space-making enzymes that are essential for the cleavage of glycosidic linkages in the PG sacculus. They are involved in a variety of physiological functions within the cell, such as turnover and recycling of PG metabolites. These PG metabolites are important virulence factors for significant human pathogens such as *Pseudomonas aeruginosa* and *Escherichia coli*. Despite the importance of LTs within the cell and their role in pathogenesis, there is a shortage of information on what mechanisms control and regulate these enzymes. This study proposes that the physiological function of the proteinaceous inhibitor, membrane-bound lysozyme inhibitor of C-type lysozyme (MliC) from *P. aeruginosa*, is to control LT activity from the same bacterium. To investigate how MliC can control LT activity, this study examined the role of MliC as an inhibitor of the soluble-derivatives of membrane-bound lytic transglycosylases A (sMltA), B (sMltB) and F (sMltF). Inhibition of sMltA and sMltF was demonstrated *in vitro* at a 1:1 equivalent molar ratio (inhibitor: LT), and inhibition of sMltB was found at a 4:1 equivalent molar ratio using the turbidimetric assay. These results provide the first experimental evidence supporting the hypothesis that the true physiological function of MliC is to control the autolytic activity of LTs of Gram-negative bacteria that do not produce O-acetylated PG.

Curriculum Vitae: Joseph completed his B.Sc. (Honours) in Microbiology, from the University of Guelph in 2015, and began his M.Sc. in the lab of Dr. Anthony Clarke in fall of 2015. After completing his studies, Joseph plans to work in the Allen-Vercoe lab with Nubiyota.