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 ***Doctor of Philosophy*** *of*

Alexander Anderson

on Wednesday, April 3rd, 2024 at 1:30p.m. (SSC 1504)

Thesis Title: Investigating the mechanism of peptidoglycan O-acetylation in Gram-negative pathogens

Examination Committee:

Dr. George van der Merwe, Molecular and Cellular Biology (Exam Chair)
Dr. Chris Whitfield, Dept. of Molecular and Cellular Biology
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Matthew Kimber, Dept. of Molecular and Cellular Biology
Dr. Michael Murphy, Dept. of Microbiology and Immunology, University of British Columbia (External Examiner)

Advisory Committee:

Dr. Anthony Clarke (Adv)
Dr. Marc Coppolino (Co-Adv)
Dr. Chris Whitfield
Dr. Cezar Khursigara

Abstract: The O-acetylation of peptidoglycan (PG) in bacteria confers a protective effect from lytic enzymes. The mechanism for PG O-acetylation by the two discrete domains of OatA is known in Gram-positive bacteria, but many questions remain about the pathway in Gram-negative bacteria. The PG *O*-acetyltransferase A (PatA) and B (PatB) are known to be necessary and sufficient for Gram-negative PG O-acetylation. Like the two domains of OatA, PatA and PatB are predicted to be an integral membrane protein and an extracytoplasmic *O*-acetyltransferase. The purpose of this thesis was to explore the mechanism of PatAB-mediated cell wall O-acetylation. To test the hypothesis that PatA and PatB function analogously to OatA, the structure and function of PatB from *C. jejuni* are described. I establish that PatB is a peripheral membrane protein and not a soluble periplasmic protein. I found the PatB periplasmic SGNH hydrolase superfamily domain represents an active *O*-acetyltransferase with broad substrate specificity. Screening of several artificial and natural candidate acetyl-intermediate species failed to support the hypothesis that a natural periplasmic species serves as an acetyl-intermediate in the pathway. The alternate hypothesis that PatA directly acetylates PatB *in vivo* was then tested. Although PatA production in *E. coli* was toxic and prevented its biochemical characterization, acetyl-tyrosine and a chemically acetylated, highly conserved PatA peptide served as an acetyl donor to PatB *in vitro*. Taken together, the data in my thesis supports a model whereby PG O-acetylation in bacteria involves a shared mechanism despite major genetic, structural, and biochemical differences in the pathway.

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Curriculum Vitae: Alexander obtained his Bachelor of Science in Biology from Wilfrid Laurier University in 2017 and in 2019, he obtained his Master of Science in Integrative Biology also from Wilfrid Laurier University. In the fall of 2019, he entered into the Ph.D. program under the supervision of Dr. Clarke and Dr. Coppolino.

Publications:

Colquhoun JM, Farokhyfar M, Anderson AC, Bethel CR, Bonomo RA, Clarke AJ, Rather PN. 2023. Collateral changes in cell physiology associated with ADC-7 β-lactamase expression in *Acinetobacter baumannii*. Microbiol. Spectr. 11(3): e04646-22 https://doi.org/10.1128/spectrum.04646-22

Anderson AC, Pimentel KN, Stangherlin S, Weadge JT, Clarke AJ. 2022. The SGNH hydrolase family: a template for carbohydrate diversity. Glycobiology 32(10): 826–848: https://doi.org/10.1093/glycob/cwac045

Anderson EM, Saji NS, Anderson AC, Brewer D, Clarke AJ, Khursigara CM. 2022. *Pseudomonas aeruginosa* alters peptidoglycan composition under nutrient conditions resembling cystic fibrosis lung infections. mSystems 7(3):e00156-22: https://doi.org/10.1128/msystems.00156-22

Colquhoun JM, Farokhyfar M, Hutcheson AR, Anderson AC, Bethel CR, Bonomo RA, Clarke AJ, Rather PN. 2021. OXA-23 beta-lactamase overexpression in *Acinetobacter baumannii* drives physiological changes resulting in new genetic vulnerabilities. mBio 12 (6), e03137-21: https://doi.org/10.1128/mBio.03137-21

Jones, CS, Anderson AC, Clarke AJ. 2021. Mechanism of *Staphylococcus aureus* peptidoglycan *O*-acetyltransferase A as an *O*-acyltransferase. Proc. Natl. Acad. Sci. 118(36): https://doi.org/10.1073/pnas.2103602118

Seepersaud R, Anderson AC, Bensing BA, Choudhury BP, Clarke AJ, Sullam PM. 2020. O-Acetylation controls the glycosylation of bacterial serine-rich repeat glycoproteins. J. Biol Chem. 296: https://doi.org/10.1074/jbc.RA120.016116.

Awards in program:

NSERC PGS-D, 2020-2022
NSERC CGS-D, 2022-2023