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*

ARJUN SUKUMARAN

On Tuesday, April 25, 2023 at 1:30 p.m. (SSC 1511)

Thesis Title: **Uncovering novel mechanisms of *Klebsiella pneumoniae* pathogenesis through mass spectrometry-based proteomics**

Examination Committee:

Dr. Michael Emes, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Jennifer Geddes-McAlister, Dept. of Molecular and Cellular Biology
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Emma Allen-Vercoe, Dept. of Molecular and Cellular Biology
Dr. Ian Lewis, Dept. of Biological Sciences, University of Calgary (External Examiner)

Advisory Committee:
Dr. Jennifer Geddes-McAlister (Advisor)
Dr. Rod Merrill
Dr. Chris Whitfield

Abstract: *Klebsiella pneumoniae* is a ubiquitous bacterial pathogen associated with nosocomial infections with the emergence of hypervirulent strains driving the appearance of community infections. Certain virulence factors of *K. pneumoniae* are well characterized; however, the acquisition of new genetic material can introduce novel virulence traits, constantly changing our understanding of infection. Bacterial pathogenesis reflects growth and survival within the host environment, evading and responding to immune cells, and interacting with other microbes that may be present. In this Thesis research, I describe the application of mass spectrometry-based proteomics to identify novel aspects influencing diverse areas of *K. pneumoniae* pathogenesis. I profiled the impact of metal (i.e., iron and zinc) availability on the proteome of *K. pneumoniae,* offering insight into nutritional immunity during infection. Here, I identified an uncharacterized protein, ChaB, with novel connections to zinc homeostasis and explored its relevance to virulence factor production. Additionally, I defined a putative role in the regulation of iron homeostasis towards Lon protease within the extracellular environment. Moreover, I investigated the interactions driving infection between *K. pneumoniae* and primary BALB/c macrophages to define promising infection-associated *K. pneumoniae* proteins and characterized their roles in bacterial growth, morphology, and virulence. Importantly, mutations to three genes encoding uncharacterized proteins displayed reduced virulence during *in vitro* and *in vivo* infection models and I discovered unique bacteria-host interacting partners that offer new insight into *K. pneumoniae* pathogenesis. Finally, I profiled a *K. pneumoniae* infection in the presence of the opportunistic fungal pathogen, *Cryptococcus neoformans,* to detect species-specific regulation and defense mechanisms within the lung environment during infection

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and I explored proteins produced by each organism. Proteomic profiling revealed suppression of *C. neoformans* in the presence of *K. pneumoniae* showcasing the complexity of microbial interactions and proposing novel strategies to combat dual infections. The implementation of quantitative proteomics analyses in these key areas of *K. pneumoniae* pathogenesis provided a global overview of bacterial adaptation and survival within distinct environments, along with interactions driving disease. Overall, this Thesis research contributes to our knowledge of *K. pneumoniae* pathogenesis and identifies proteins as putative novel targets for anti-virulence discovery.

Curriculum Vitae: Arjun completed his Bachelor of Science (Hons.) at the University of Toronto in 2014. He completed his Master of Science in the Biology department at Western University under the joint supervision of Dr. Sangeeta Dhaubhadel and Dr. Denis Maxwell in 2016. He began his PhD at the University of Guelph in September 2018 under the supervision of Dr. Jennifer Geddes-McAlister.

Awards: Ontario Graduate Scholarship (2021); Thermo Fisher Scientific CSMS Travel Award (2021); Queen Elizabeth II Graduate Scholarship in Science and Technology (2020); CSM Travel Award (2019).

Publications: **Sukumaran, A.[1]**, Ball, B.[1], Krieger, J. R., & Geddes-McAlister, J. (2022). Cross-kingdom infection of macrophages reveals pathogen-and immune-specific global reprogramming and adaptation. mBio, 13(4), e01687-22. [1]Authors contributed equally.

Sukumaran, A. & Geddes-McAlister, J. (2022). Proteomic profiling of the interplay between a bacterial pathogen and host uncovers novel anti-virulence strategies. Methods Mol Biol: Proteomics in Systems Biology. (Invited submission; Book Chapter).

Ball, B., Woroszchuk, E., **Sukumaran, A.**, West, H., Afaq, A., Carruthers-Lay, D., ... & Geddes-McAlister, J. (2021). Proteome and secretome profiling of zinc availability in *Cryptococcus neoformans* identifies Wos2 as a subtle influencer of fungal virulence determinants. *BMC microbiology*, *21*(1), 1-16.

Sukumaran, A., Woroszchuk, E., Ross, T., & Geddes-McAlister, J. (2021). Proteomics of host–bacterial interactions: new insights from dual perspectives. *Canadian Journal of Microbiology*, *67*(3), 213-225.

Sukumaran, A., Pladwig, S., & Geddes-McAlister, J. (2021). Zinc limitation in *Klebsiella pneumoniae* profiled by quantitative proteomics influences transcriptional regulation and cation transporter-associated capsule production. *BMC microbiology*, *21*(1), 1-15.

Ball, B., **Sukumaran, A.**, & Geddes-McAlister, J. (2020). Label-Free Quantitative Proteomics Workflow for Discovery-Driven Host-Pathogen Interactions. *Journal of Visualized Experiments: Jove*, (164).

Muselius, B.[1], **Sukumaran, A.**[1], Yeung, J., & Geddes-McAlister, J. (2020). Iron limitation in *Klebsiella pneumoniae* defines new roles for Lon protease in homeostasis and degradation by quantitative proteomics. *Frontiers in Microbiology*, *11*, 546. [1]Authors contributed equally.

Sukumaran, A., Coish, J. M., Yeung, J., Muselius, B., Gadjeva, M., MacNeil, A. J., & Geddes-McAlister, J. (2019). Decoding communication patterns of the innate immune system by quantitative proteomics. *Journal of leukocyte biology*, *106*(6), 1221-1232.