



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

STEVAN CUCIĆ

on Friday, September 20th, 2024 at 9:30a.m. (SSC 2315)

Thesis Title: Phage-mediated Dispersal of *Listeria monocytogenes* Biofilms and Multi-omic Characterization of a Lytic *Listeria* Phage

Examination Committee:

Dr. Matthew Kimber, Molecular and Cellular Biology (Exam Chair)
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Jennifer Geddes-McAlister, Dept. of Molecular and Cellular Biology
Dr. Emma Allen-Vercoe, Dept. of Molecular and Cellular Biology
Dr. Matthew Gilmour, Quadram Institute of Bioscience, Norwich, UK (External Examiner)

Advisory Committee:

Dr. Cezar Khursigara (Co-Adv)
Dr. Hany Anany (Co-Adv)
Dr. Jennifer Geddes-McAlister
Dr. Rebecca Shapiro

Abstract: *Listeria monocytogenes* is a pathogenic foodborne bacterium capable of causing invasive listeriosis, a disease with a high mortality rate. This bacterium can grow at low temperature and water activity, making it challenging to control in food processing environments. Moreover, *L. monocytogenes* can form biofilms and colonize pre-formed biofilms, which contributes to its persistence by providing protection from industrially used sanitizers. Bacteriophages (phages) have evolved to infect bacteria in biofilms. Consequently, there is interest in developing phage-based biosanitizers to control pathogenic bacteria in food processing environments. We genomically and phenotypically characterized a panel of nine related lytic *Listeria* phages to assess their host range, virulence in liquid culture and ability to erode axenic *L. monocytogenes* biofilms. One of the phages, called CKA15, that showed relatively higher activity was further tested for its ability to inactivate mature biofilms, to adsorb to its host under application-relevant conditions as well as to degrade axenic *L. monocytogenes* biofilms grown under simulated dairy processing conditions (SDPC). CKA15 caused a 2-log reduction in sessile *L. monocytogenes* counts under SDPC. To gain insight into this phage regarding the proteins comprising the mature phage particles, the operon structure of the phage genome as well as the infection program of the phage and host response to infection, we performed proteogenomic analysis of purified phage particles and transcriptomic analysis of phage-infected bacteria using two different RNAseq techniques. We detected 29 putative phage particle-associated proteins. The global detection of phage transcriptional start sites, promoter motif analysis, sequence analysis of phage gene products, and time-resolved transcript abundance during infection enabled us to propose a model wherein the switch from early to late gene transcription is mediated by a switch in recognition by the host RNA polymerase from SigA consensus

sequence early promoters to late promoters involving phage-encoded sigma and anti-sigma factors, which are expressed in the middle stages of the phage replicative cycle. This work demonstrates the potential utility of a strictly lytic *Listeria* phage in inactivating *L. monocytogenes* biofilms in dairy environments and contributes to elucidating replication and assembly of a P100-like phage.

Curriculum Vitae: Stevan received his BSc (honours) in Microbiology from the University of Guelph in 2011. In 2017, he also received his MSc in Food Science from the University of Guelph. In the fall of 2018 he entered the PhD program under the joint supervision of Dr. Khursigara and Dr. Anany.

Publications: El-DougDoug, N. K., Cucic, S., Abdelhamid, A. G., Brovko, L., Kropinski, A. M., Griffiths, M. W., & Anany, H. (2019). Control of Salmonella Newport on cherry tomato using a cocktail of lytic bacteriophages. *International journal of food microbiology*, 293, 60-71.

Martinez-Soto, C. E., Cucic, S., Lin, J. T., Kirst, S., Mahmoud, E. S., Khursigara, C. M., & Anany, H. (2021). PHIDA: a high throughput turbidimetric data analytic tool to compare host range profiles of bacteriophages isolated using different enrichment methods. *Viruses*, 13(11), 2120.

Cucic, S., Kropinski, A. M., Lin, J., Khursigara, C. M., & Anany, H. (2022). Complete genome sequence of a jumbo bacteriophage, Escherichia phage vB_EcoM_EC001. *Microbiology Resource Announcements*, 11(2), e00017-22.

Cucic, S., Ells, T., Guri, A., Kropinski, A. M., Khursigara, C. M., & Anany, H. (2024). Degradation of *Listeria monocytogenes* biofilm by phages belonging to the genus Pectentumvirus. *Applied and Environmental Microbiology*, 90(3), e01062-23.