DEPARTMENT OF AGRICULTURAL, FOOD AND NUTRITIONAL SCIENCE

PhD Thesis Seminar

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Date:	**Tuesday, September 19, 2023**
Time:	**9:15 am**
Location:	**4-10C Agriculture/Forestry Centre**
Title:	**Understanding host-microbiome interactions and influence on STEC colonization in cattle using integrated omics**

ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) is the major foodborne pathogen in humans with Shiga toxin 1 (stx1) and 2 (stx2) being the main virulence factors. Cattle are the major reservoir of STEC with shedding $>10^4$ CFU/g STEC being super shedders which is the critical disseminator for STEC. The rectal anal junction (RAJ) was identified as the primary colonization site of STEC and previous studies revealed that both fecal and rectal mucosal microbiota were related to STEC colonization in RAJ. To date, the mechanisms of stx in STEC affecting host-microbial interactions remain unidentified. This thesis aimed to identify how host and fecal/mucosal microbiota respond to stxs in STEC and STEC colonization. Study 1 performed an epidemiological survey to reveal the expression (RNA) of *stx1* and *stx2* in STEC. Besides, the Spearman correlation revealed that the *stx2* expression was related to expressions of host immune genes previously reported to be downregulated in SS including *MS4A1*, *CCL21*, *CD19*, and *LTB*. The random forest model and Boruta method further identified that *MS4A1* was the most predictive gene for the prediction of the *stx2* expression, suggesting that the *stx2* expression in STEC related to host immunities. Study 2 performed amplicon sequencing to characterize differences in rectal digesta microbial profiles and interactions using samples collected from steers in which *stx2* was not expressed (Stx2- group) and those with *stx2* expressed (Stx2+ group). Although microbial diversities and similarities did not differ between two groups, microbial interactions revealed by microbial networks were remarkably different with group-specific microbes being the most connected taxa within the microbial network for each group. These results suggested that the *stx2* expression contributed to the inherently different microbial community structures even when their diversity and compositions were comparable to the Stx2- group. Study 3 identified variations of host transcriptomes in veal calves challenged with STEC O157 with the ability to lack stx2a (WT group) or possess stx2a (RE group) using rectal mucosa samples collected pre- (T1) to 7 days (T2) and 26 days post-challenge (T5). The stx2a is a subtype of stx2 and is critical for STEC pathogenicity. A total of 214 downregulated differential expression genes (DEGs) were identified in WT-T2 compared to RE-T2, while a total of 152 upregulated DEGs and 45 GO terms were identified for both WT-T5 and RE-T5. The functional analysis revealed that the challenge in WT induced decreased functions at T2 relative to extracellular regions and tissue barrier integrity while these functions were then enhanced at T5. For RE, no functional variations were found at T2 and only enhanced aforementioned functions were identified at T5, suggesting that the *stx2a* expression in STEC could cause differed host responsive patterns. In study 4, cDNA amplicon sequencing was performed to characterize how active rectal mucosa microbial profiles, interactions, and assembly as well as host-microbial interactions related to strain-specific STEC O157 colonization. The rectal mucosa microbial diversities were not affected by the presence of *stx2a* in STEC. Instead, the dynamics of microbial interactions and assembly patterns differed in response to strain-specific STEC O157 colonization. The relative abundance of *Paeniclostridium* and *Gallibacterium* were identified as connectors in microbial networks and specialists in microbial assembly. Host immune responses varied post-challenge with B-cell and T-cell signaling receptor pathways, antigen processing and presentations being upregulated in both WT and RE. The beneficial microbes dominated interactions with host immune gene expressions pre-challenge, while the opportunistic pathogen *Paeniclostridium* drove interactions with host immune genes post-challenge and such relationship depended on the production of stx2a. In summary, this thesis provides knowledge of host-microbial interactions in response to *stx* gene expression and STEC colonization. Our findings suggest that STEC colonization and *stx* gene expression could be systematically attributed to differences in genetic variations, host responses, and fecal/mucosal microbiota.