NANYANG TECHNOLOGICAL UNIVERSITY

School of Biological Sciences

Reg. No. 200604393R

Research Theme: Infection and Immunity

Research Project Title: Formation and function of membrane lipid microdomains in *Enterococcus faecalis*

Principal Investigator/Supervisor: Assoc/Prof Kimberly Kline (SBS/SCELSE)

Co-supervisor/ Collaborator(s) (if any):

Project Description

a) Background:

The Gram-positive Enterococci are commensal inhabitants of the gastrointestinal tract, as well as opportunistic nosocomial pathogens associated with endocarditis, wound infections, and urinary tract infections. Virulence factor biogenesis in *Enterococcus faecalis* occurs at discrete foci that are coordinated, in part, by microdomains of anionic lipids. These microdomains are akin to "lipid rafts" in eukaryotic cells. Bacterial microdomains can be targeted by cationic antimicrobial agents to disrupt virulence factor assembly sites, but the bacterium has evolved strategies to protect these focal sites. One protective mechanism involves increasing the charge of anionic membrane microdomain lipids via lysinylacylation of phosphatidylglycerol (PG) to create LPG. Surprisingly, we have found that altering the bacterial LPG state also significantly alters overall cellular lipid homeostasis. Furthermore, *E. faecalis* mutants that are resistant to the cationic antibiotic daptomycin have substantially lower levels of PG and LPG levels. Since LPG levels in these mutants are lower, daptomycin resistance is likely due to the reduction in PG. Together, our findings to date suggest that anionic membrane microdomains are functional for virulence factor assembly and may serve as an Achilles's heal for antimicrobial targeting.

b) Proposed work:

While we have identified several *E. faecalis* factors that localize to anionic lipid microdomains, we do not yet know their function. Thus, the goal of this project is to determine how microdomain-localized proteins function in microdomain biogenesis, stability, and function in coordinating virulence factor assembly. Specifically, this project will answer the following questions: What is the lipid and protein composition of membrane microdomains at different stages of the bacterial cell cycle? How do microdomain-associated proteins affect cell physiology. Must microdomain-associated proteins always be located within microdomains for their function? Methods including fluorescent microscopy, mass spectrometry and lipidomics, as well as fundamental biochemistry and molecular biology approaches will be used in this project.

Supervisor contact:

If you have questions regarding this project, please email the Principal Investigator:
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