ABSTRACT

Though most of the microbes are unicellular, yet they display several complex social traits like communication, swarming motility, regulation of coordinated gene expression, etc. similar to the higher multicellular organisms. They compete or cooperate with neighboring microbes for limited space and nutrients to ensure survival. During the course of evolution, microbes have evolved several different strategies to communicate and compete. To restrict the growth of competitors and promote kin selection, microbes secrete several proteinaceous and non-proteinaceous toxic molecules. Polymorphic Toxin Systems (PTS) belong to one such diverse and widespread family of toxins produced by bacteria that is involved in the inter-bacterial competition. Contact-Dependent growth Inhibition (CDI) systems have also been classified under PTS. In the present thesis we describe the structural, functional, and biophysical characteristics of CDI system from uropathogenic *Escherichia coli* strain 536 (UPEC536) and a member of Pfam PF04070 family of PTS from *B. subtilis* ATCC 6633.

CDI is a recently discovered phenomenon where CDI^+ bacterial strain (inhibitor) inhibits the growth of CDI^- strain (target) by the direct cell to cell contact. CDI is mediated by *cdiBAI* gene cluster where CdiB facilitates the export of CdiA, an exotoxin, on the cell surface and CdiI acts as an immunity protein to protect CDI^+ cells from auto-inhibition. CdiA-CT, the C-terminal region of the toxin CdiA, from UPEC536 is a latent tRNase that requires binding of a biosynthetic enzyme CysK (O-acetylserine sulfyhydrylase) for activation in the target cells. CdiA-CT can also interact simultaneously with CysK and immunity protein, CdiI, to form a ternary complex in UPEC536. How CdiA-CT is activated by CysK, and neutralized by CdiI was not known. Also, the role of CysK in the ternary complex in inhibitor cells, UPEC536, was also not clear. The Chapter 2 of this thesis describes the hydrodynamic, thermodynamic and kinetic parameters of binary (CdiA-CT/ CdiI, CdiA-CT/ CysK) and ternary complexes (CdiA-CT/ CdiI/ CysK)using Analytical Ultracentrifugation (AUC), Isothermal Titration Calorimetry (ITC) and Surface Plasmon Resonance (SPR) respectively, to investigate the role of CysK in UPEC536. We report that CdiA-CT binds CdiI and CysK with nanomolar range affinity. We further report that binding of CysK to CdiA-CT improves its affinity towards CdiI by ~40 fold resulting in the formation of a more stable complex with over ~130 fold decrease in dissociation rate. Thermal melting experiments also suggest the role of CysK in stabilizing CdiA-CT/CdiI complex as T_m of the binary complex shifts ~10 °C upon binding CysK. Hence, CysK acts a modulator of CdiA-CT/CdiI interactions by stabilizing CdiA-CT/CdiI complex and may play a crucial role in

preventing auto-inhibition in UPEC536. So, we propose that CysK plays dual role in UPEC536 CDI system. This study reports a new moonlighting function of a biosynthetic enzyme, CysK, as a modulator of toxin/immunity interactions in UPEC536 inhibitor cells. CysK also provide thermal stability to CdiA-CT which may probably help in substrate binding and activating toxin. To further gain structural insights to unravel the mechanism of CdiA-CT activation by CysK and toxin neutralization by CdiI we created several constructs, purified binary, and ternary complexes and subjected to extensive crystallization trials. We successfully crystallized ternary complex and collected data at 3.3 Å resolution. While we were optimizing crystals for phasing experiments Goulding's group from solved the binary and ternary complex structures. Using these models, we solved our ternary complex structure. The detailed crystallization, data collection, and structural analysis is described in Chapter 2.

Though widespread, the PTS from gram-positive bacteria has been the least studied. Chapter 3 describes the functional, biophysical and structural characterization of YeeF/YezG, a PTS from *B. subtilis*. We show that expression of YeeF-CT, the C-terminal toxic domain of YeeF, is toxic and causes growth inhibition in *E. coli*. Co-expression of YezG with YeeF-CT can rescue cells from the YeeF-CT-mediated toxicity; hence, YeeF-CT/YezG forms a toxin/immunity protein pair. Our data suggest that YeeF-CT is a metal-dependent nonspecific DNase having a rare property of activation by different metal ions. We also report YeeF-CT as a unique bacterial toxin which can form a ternary complex with YezG and DNA indicating that YezG and DNA can bind YeeF-CT at non-overlapping sites. This data suggests that YezG acts as an "exosite" inhibitor of YeeF-CT. Experiments with purified YeeF-CT suggests that toxin can enter target cells and cause DNA damage leading to growth arrest. It also displays activity against both gram-positive and gram-negative bacteria, though it is more effective against gram-positive bacteria. Our biophysical experiments (SAXS, AUC, ITC, SPR and BLI) suggest that YeeF-CT is a homodimer which binds YezG with nanomolar range affinity to form a 2:2 heterotetramer in solution. The present study will provide new insights into the functional and structural diversity of polymorphic toxins and will lay foundation to further investigate the unique properties of PTS/CDI systems.