Systems and Synthetic Biology Seminar mini workshop

3/14 (Awd) 16:00 - 17:30 D105 seminar room

Structural and functional studies on bacterial proteins

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My lab focuses on the structural studies on bacterial proteins, including the transcriptional regulators, enzymes and virulence factors that are essential for the pathogenesis. The primary techniques used in my lab including protein crystallography and small-angle X-ray scattering (SAXS) that offer great advantages to facilitate our fundamental understanding of the structure-function relationship of the molecules. In this talk, I will first briefly introduce the technologies for structural determination of biological molecules. Then I will present our results from two projects that combines crystallography and SAXS to resolve the structures of 1) a bacterial transcriptional regulator Rgg from *Streptococcus pyogenes*; 2) the structure and substrate specificity of the Clostridium difficile sortase enzyme essential for pathogenesis for its role in displaying surface proteins to bacterial cell wall. My talk will focus on how the three-dimensional structures contribute to our knowledge of molecular actions at atomic level and, how the information of molecular basis provides insights into therapeutic development against infectious diseases.

Keywords: protein crystallography; small-angle X-ray scattering; transcriptional regulators; substrate specificity; sortase.

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Association between DNA methylation and pathogenicity in urinary tract

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E. coli comprised of strains showing different phenotypes, which are non-pathogenic, enterohemorrhagic, uropathogenic, and so on. Such phenotypic features between strains are resulted from different genomic structure. From genome comparison of many *E. coli* strains, bacterial genome shows mosaic structure and is comprised of core-genome and accessary-genome, which involves in the common feature of the species and the phenotypes specific to the strains, respectively. The accessary genes characterizing the strain are often clustered (genomic island) and acquired by horizontal gene transfer.

Restriction-modification in bacteria is an immune system to exclude foreign DNA. Type IV restriction enzymes (T4RE) specifically digests methylated DNA sequence. A T4RE and a DNA methyltransferase (DNMT), which the recognition sequences are overlapped, can not co-exist in a cell, because their genomic DNA would be methylated and digested. Then, we investigated whether the conflict led lethality affected the horizontal gene transfer and genomic evolution using uropathogenic *E. coli* as a model.

Plasmid expressing a T4RE (McrBC) is introduced to 83 of clinically isolated urinary tract infectious (UTI) *E. coli*, and 50 strains of *E. col*i isolated from fecal. The frequency of lethality by the conflict is significantly higher in the UTI isolates than fecal isolates. It suggests that the distribution of DNMT shown the conflict is higher in UTI E. coli. Nine DNMT genes were specifically identified, and the conflict was confirmed with cloned DNMTs. We also performed pathogenicity assay using Caenorhabditis elegans to investigate whether the DNA methylation involved in level of pathogenicity of the clinical isolates. However, there are no significant association between the existence of DNMT and pathogenicity level in the model. These results implied that the DNMTs involved in horizontal gene transfer to acquire virulence genes, but did not involve to increase the pathogenicity in the UPEC strains.

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Identification of mobilizable plasmids that benefit bacterial pathogens during infections

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Antibiotic-resistant transmissible plasmids encoding factors facilitating pathogenic bacteria to cause infections are emerging public health threats. Such plasmids can effectively transfer among bacteria of different species through conjugation. Accordingly, the pathogenic factors as well as the antibiotic resistance encoded in the plasmids can be rapidly and broadly disseminated in bacterial populations. In addition to the antibiotic resistance, these pathogenic traits confer extra selection advantage for their pathogenic bacterial hosts during the course of infections. Identification and characterization of such plasmids will contribute to developing novel strategies against the infections caused by their pathogenic bacterial hosts.

This study was initiated from a screen for the ampicillin-resistant transmissible plasmids that benefit their bacterial hosts during infections. These plasmids in the *Klebsiella pneumoniae* and Escherichia coli clinical isolates were transferred to the commensal *E. coli* strain MG1655 strR through conjugation. We obtained 8 and 10 trans-conjugants harboring plasmids from the *E. coli* and *K. pneumoniae* clinical isolates. Then the mixture of the trans-conjugants and MG1655 strR were subjected to the mouse air pouch and urinary tract infection (UTI) models to determine which trans-conjugants show higher levels of survival in the animals in comparison with MG1655 strR. One trans-conjugant MG1655 strR/ EC23 showed significantly higher bacterial counts in the mouse infection models. MG1655 strR/ EC23 acquired more than one plasmid through the conjugation with an *E. coli* clinical isolate. Among the plasmids, pEC23-3 was identified to be involved in providing conjugation ability, while pEC23-4 was shown to be able to confer its bacterial host competitive advantage during infection. pEC23-4 contains the colicin K and the mobilizable gene clusters. We confirm that the colicin K gene cluster confers *E. coli* competitive advantage during the course of UTIs, systemic infections, and intestinal colonization.

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