Identification and characterization of bacterial small RNAs in regulating \textit{Erwinia amylovora} pathogenesis (Oral presentation)

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Aim:
Fire blight, caused by bacterial pathogen \textit{Erwinia amylovora}, is the most serious disease currently limiting apple and pear production in the United States. To successfully cause diseases in host plants, \textit{E. amylovora} utilizes multiple regulatory systems to modulate the expression of virulence factors at the early and late stage of infection. Bacterial small RNAs (sRNAs) are non-coding regulatory RNA molecules that bind to and regulate the expression of target mRNAs. sRNAs have been found to control multiple virulence determinants in many animal bacterial pathogens. However, there are few reports of sRNAs in the virulence regulation in plant bacterial pathogens. In this work, we are trying to determine the role of sRNAs in virulence regulation in the fire blight pathogen \textit{E. amylovora}.

Goals:
1. Use RNA-seq to identify unannotated bacterial small RNAs in \textit{E. amylovora}, confirm their expression, and annotate their genetic locations on the chromosome of \textit{E. amylovora}.
2. Screen for virulence-regulating sRNAs and study the mechanisms how they regulate virulence in \textit{E. amylovora}

Results and conclusions:
42 novel sRNAs were identified and annotated in the genome of \textit{E. amylovora}. The expression and sizes of 14 sRNAs were confirmed by Northern blot, and the boundaries of 7 sRNAs were confirmed by 5’ RACE assay. To understand the virulence regulation of these sRNAs, deletion mutants of 23 sRNAs were constructed, and the virulence as well as the expression of various virulence factors of these mutants were compared with the wild type \textit{E. amylovora}. The mutation of three sRNAs, ArcZ, RprA and Hrs21, lead to greatly reduction in virulence compared to \textit{E. amylovora}. Three sRNAs, ArcZ, Hrs6, and OmrAB were identified as positive regulators of motility. ArcZ positively regulate the type III secretion system. Hrs6 and OmrAB repress the amylovoran production whereas ArcZ activates the amylovoran production. Detailed regulation of motility by ArcZ was also characterized. We demonstrated that ArcZ control bacterial motility through regulating the master flagellar regulator FlhDC and the sensor kinase of a two component signal transduction system RcsC. A physical interaction between ArcZ RNA and \textit{flhDC} mRNA was observed, and the key nucleotides affecting the translational repression of \textit{flhDC} were characterized. These results suggest that multiple bacterial sRNAs are required for the virulence regulation in fire blight pathogen \textit{E. amylovora}. These sRNAs could be intriguing targets for the development of fire blight control using RNA silencing.