Investigation of the transcriptional landscape and RNA biology of *Salmonella* Typhimurium plasmids

PhD Thesis presented by Sathesh Kumar Sivasankaran
To the Department of Microbiology at Trinity College Dublin
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Plasmids are extra-chromosomal, self-replicating genetic elements found in all kingdoms of life. Plasmids play a major role in bacterial adaptation to environmental changes and contribute to the overall plasticity of the bacterial genome. The accessory functions carried by plasmids include antibiotic resistance and virulence factors, making them particularly important for medical bacteriology.

As one part of this project, a bioinformatics pipeline was developed for the efficient analysis of RNA-Seq data. Overall, 106 RNA-Seq libraries and ~1.75 billion cDNA reads were processed using the analysis pipeline. The data for visualisation were generated using custom-made Perl scripts, and relative transcript abundance was determined with the Transcripts Per Million approach. The RNA-Seq pipeline has been used extensively and successfully to analyse RNA-Seq data generated from different bacterial species.

A second aspect of this project involved investigation of the RNA biology of the three plasmids of *Salmonella enterica* serovar Typhimurium. A total of 53 RNA-Seq libraries generated from 23 distinct infection-relevant environmental conditions were used to generate a robust picture of transcription of *S*. Typhimurium plasmids. Eight dRNA-Seq datasets generated from four growth conditions identified 162 TSS on the plasmids, including 59 primary and 56 antisense TSS. Of the 120 TSS expressed in ESP, 86 were bound with the 'housekeeping' transcriptional factor RpoD. The RNA-Seq data identified 39 antisense RNAs (asRNAs) and a pSLT$^{4/74}$-encoded sRNA, STncP1-1. Identification of seven asRNAs within the *pef* locus made this region a hot spot for antisense transcription. The discovery of numerous antisense transcripts in *Salmonella* plasmids suggests that antisense transcription may be a common feature of plasmids.

A putative role of STncP1-1 in *Salmonella* virulence was identified using macrophage infection experiments and mouse models. A total of 72 candidate targets of plasmid-encoded sRNA/asRNA-mediated regulation in *S*. Typhimurium were found. However, confirmatory experiments were inconclusive.

A unique expression profile of plasmid-encoded genes across 23 environmental conditions was identified. The plasmid core-functional genes were expressed in most of the suite conditions, including genes involved in replication, partitioning and stability of the plasmids. The genes encoding virulence traits were only expressed in growth conditions relevant to the intracellular survival of the bacterium, such as the *spv* locus. It was found that genes with a similar function had similar expression profiles, e.g., partitioning systems from pSLT$^{4/74}$ and pCol1B$^{4/74}$, and the *spv* locus clustered with SPI-2. In contract, the plasmid post-segregational killing systems encoded by pSLT$^{4/74}$ and pCol1B$^{4/74}$ showed distinct expression patterns, even though they perform a similar function in plasmids. The highly complex network of interactions between plasmids and chromosome of *Salmonella* at the regulatory level was identified with a series of mutants that lack known regulators, including transcription factors, global regulators and SPI-1 and SPI-2 associated regulators.