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On the application of DNA pyrosequencing in pathogenomics and food safety

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The development of DNA pyrosequencing approaches has enabled researchers to study and understand microorganisms from deeper perspectives. DNA sequencing technologies has evolved through first, second (next) and currently stands at third generation sequencing platforms. Since bacteria can be transmitted from wider environment to animals and humans, they encounter diverse environments which include food, water, plant surfaces, extra and intracellular phases of infection in eukaryotic hosts. An intricate transcriptional network has evolved to respond to the wide variety of environmental signals and control the right time/right place expression of genes. We used deep sequencing of cDNA libraries (RNA-seq) to understand the transcriptional landscape of *Salmonella typhimurium* during survival in 23 infection related *in vitro* conditions and mouse macrophages as an *ex vivo* model. Our analysis yielded data on the simultaneous gene expression profiling of all genes present in the genome of *Salmonella typhimurium*. In addition, we used a technology called dRNA-seq to profile the expression of different promoters during survival of the bacterium in different conditions. Reduction in water activities inhibits many bacteria and desiccation is therefore a very traditional method of food preservation. We were also interested in understanding the intricate mechanisms behind survival of bacteria in desiccated conditions. However, neonatal pathogens like *Cronobacter sakazakii* are capable to survive desiccation conditions. We used RNA-seq to understand the genetic mechanism underlying desiccation survival in *Cronobacter sakazakii*. DNA deep sequencing not only helps us understand how transcriptional alterations aid survival of bacteria in different conditions but also enables taxonomic identification of bacteria in complex microbiomes. We sampled low/medium/high care areas within a powder infant formula industry and used 16S rDNA sequencing/metagenomic/metatranscriptomic approaches to understand the composition and interactions between different bacterial communities within each area. Overall, this lecture will explore the different possibilities of DNA pyrosequencing in understanding pathogenomics and food safety.

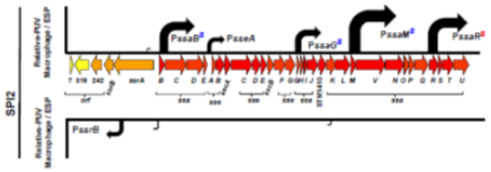

Figure 1. The transcriptional architecture of *Salmonella* Pathogenicity Island 2 (SPI2) during intra-macrophage survival of *Salmonella*. The arrows represent genes, their orientation and length. The colour of the gene represents their intra-macrophage expression with red colour showing high up-regulation and yellow showing no change in expression. The curved arrows represent transcriptional start sites expressed within SPI2.

Recent Publications

1. Cao Y, Fanning S, Proos S, Jordan K and Srikumar S (2017) A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. *Front Microbiol*; 8: 1829.
2. Anvarian A H, Cao Y, Srikumar S, Fanning S and Jordan K (2016) Flow cytometric and 16S sequencing methodologies for monitoring the physiological status of the microbiome in powdered infant formula production. *Front Microbiol*; 7: 968.

Biography

Shabarinath Srikumar has his expertise in investigating pathogenomics of food borne pathogens. He has widely used next generation sequencing approaches in characterizing the transcriptional landscape of the pathogen during survival in a variety of *in vitro* and *in vivo* conditions. Presently, he has focused on the utilization of next/third generation sequencing approaches in the taxonomic characterization of bacteria from complex microbiomes.

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