133. The small RNome of *Clostridium acetobutylicum* that orchestrates metabolite stress response

Keerthi P. Venkataramanan[1,2,*] (venkatkp@udel.edu), Shawn W. Jones[1,2] Kevin P. McCormick[2,3], Sridhara G. Kunjeti[2,4], Matthew T. Ralston[2,5], Blake C. Meyers[2,4] and **Eleftherios T. Papoutsakis**[1,2]

[1]Department of Chemical and Biomolecular Engineering, University of Delaware. [2]Delaware Biotechnology Institute, University of Delaware. [3]Department of Computer and Information Sciences, University of Delaware, Newark. [4]Department of Plant and Soil Sciences, University of Delaware. [5]Center of Bioinformatics and Computational Biology, University of Delaware, Newark, DE 19701, USA.

Project Goal: The objective of this project is to engage next generation sequencing technology for the identification the differential expression of small non-coding regulatory RNAs (sRNAs). The outcome of this study will result in the understanding the complexity, paradigm and importance of toxic metabolite stress in *C. acetobutylicum*.

Abstract:
Regulatory small non-coding RNAs (sRNA) have been identified in several Gram$^+$ and Gram$^-$ prokaryotes and are emerging as major, previously unrecognized, components of the cellular regulatory network and structural machinery, several possessing their own regulons. Among those, few sRNAs have been reported as being involved in toxic metabolite stress, mostly in Gram$^-$ prokaryotes, but hardly any in Gram$^+$ prokaryotes and still, their role at the systems level remains poorly understood, especially so in metabolite and general stress responses. In the important genus of *Clostridium*, which is of major importance to pathogenesis, human physiology, the carbon cycle and biotechnological applications, very few sRNAs have been so far identified.

Using RNA deep sequencing (RNA-seq) we examined the sRNome of *C. acetobutylicum* in response to the native but toxic metabolites, butanol and butyrate. 50% of the RNA-seq reads mapped to genomic DNA outside annotated ORFs, thus demonstrating the richness and importance of the small RNome. Using the 113 sRNAs we had previously computationally predicted [1] together with annotated mRNAs, we set metrics for reliably identifying sRNAs from RNA-seq data, thus discovering 46 additional sRNAs. Under metabolite stress, these 159 sRNAs displayed distinct expression patterns, a select number of which was verified by Northern analysis. We identified stress-related expression of sRNAs affecting transcriptional (6S & S-box, *solB*) and translational (*tmRNA* & *SRP-RNA*) processes, and 65 likely targets of the RNA chaperone Hfq. Our results support an important role for sRNAs in toxic-metabolite stress response [2].

This is the first study to elucidate the role of sRNAs in clostridial response to metabolite stress and is essential for understanding the complexity of the regulatory network that underlies the metabolite-stress response, whether related to normophysiology, pathogenesis or biotechnological applications and how that network can be engineered for practical applications to produce chemicals and fuels or for remediation processes.

References

1. Chen, Y., et al., *Small RNAs in the genus Clostridium.* MBio, 2011. **2**(1): p. e00340-10.
2. Venkataramanan, K.P., et al., *The Clostridium small RNome that responds to stress: the paradigm and importance of toxic metabolite stress in C. acetobutylicum.* BMC Genomics, 2013. **14**: p. 849.

Acknowledgement: *This work was supported by the genomic sciences program grant from Department of Energy, USA (grant#DESC0007092).*