185. The Core Regulons Orchestrating the Response of *Clostridium acetobutylicum* to Butanol and Butyrate Stress

Qinghua Wang[1,2,3,#,*] (wangq@dbi.udel.edu), Keerthi P Venkataramanan[1,4,#], Hongzhan Huang[1,2,3], **Eleftherios T Papoutsakis**[1,4], and **Cathy H Wu**[1,2,3]

[1]Delaware Biotechnology Institute, University of Delaware, Newark; [2]Center for Bioinformatics and Computational Biology, University of Delaware, Newark; [3]Department of Computer and Information Sciences, University of Delaware, Newark; and [4]Department of Chemical and Biomolecular Engineering, University of Delaware, Newark

http://clostress.org/

Project Goal: The objective of this project is to develop integrated, predictive models of the metabolic and regulatory networks of the metabolite stress response in solventogenic clostridia using enabling systems-biology approaches. Clostridia are Gram$^+$, obligate anaerobic, endospore forming bacteria of major importance to fermentative biofuel production. We focus on understanding and modeling the stress response of *Clostridium acetobutylicum* to two important toxic metabolites: butanol and butyrate. This is a problem of major importance not only in clostridial biotechnologies, but in all microbial systems of interest to DOE for bioenergy production. Coupling a large set of transcriptome data we generated with genome-scale regulon analyses, we have successfully constructed a stress response network model. The bioinformatics and systems biology efforts are applicable to a broad set of microorganisms of interest to the production of biofuels and chemicals from renewable sources.

Organisms of the genus *Clostridium* are Gram$^+$ endospore formers of great importance in human normo- and pathophysiology, and biofuel and biorefinery applications. Exposure of *Clostridium* organisms to chemicals, in particular toxic metabolites, is ubiquitous in both natural (such as in human gut) and engineered environments, engaging both the general stress response as well as specialized programs. Yet, despite its fundamental and applied significance, it remains largely unexplored at the systems level.

In this study, we generated a total of 96 individual sets of high-resolution microarray data examining the transcriptional changes in *C. acetobutylicum*, a model *Clostridium* organism, in response to three levels of chemical stress from the native metabolites, butanol and butyrate. We identified 164 significantly differentially expressed transcription regulators and detailed the cellular programs associated with general and stressor-specific responses, many previously unexplored. Pattern-based, comparative genomic analyses enabled, for the first time, to construct the stress-responsive regulons in *C. acetobutylicum* under butanol and butyrate stress. Notably, the regulons and binding motifs of the stress-related transcription factors (HrcA, CtsR, LexA, Rex and PerR) were defined together with those controlling stress-responsive amino acid and purine metabolism (ArgR, HisR, CymR and PurR).

Using a large set of high-throughput temporal gene expression data in combination with genome-scale regulon analyses, we have successfully built a stress response network model integrating important players for the general and specialized metabolite stress response in *C. acetobutylicum*[1]. Since the majority of the transcription factors and their target genes are highly conserved in other organisms of the *Clostridium* genus, this network would be largely applicable to other *Clostridium* organisms. The network informs the molecular basis of *Clostridium* responses to toxic metabolites in natural ecosystems

and the microbiome, and will facilitate the construction of genome-scale models with added regulatory-network dimensions to guide the development of tolerant strains.

References

1. Wang, Q., Venkataramanan, K. P., Huang, H., Papoutsakis, E. T., and Wu, C. H. (2013) Transcription factors and genetic circuits orchestrating the complex, multilayered response of *Clostridium acetobutylicum* to butanol and butyrate stress, BMC Syst Biol 7, 120.

The work was supported by the genomic science grant from Department of Energy, USA (grant # DE-SC0007092).

[#] Authors contributed equally