134. Experimental Systems-Biology Approaches for Clostridia-Based Bioenergy Production: The Metabolite Stress-Response System in Solventogenic Clostridia

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Project Goals: The objectives of this project are to engage enabling experimental systems-biology approaches to support the development of integrated, predictive models of the metabolic and regulatory networks underlying the metabolite stress response in solventogenic clostridia. Clostridia are Gram$^+$, obligate anaerobic, endospore-forming bacteria of major importance to fermentative biofuel production. Here, we focus on understanding and modeling the stress-response of *Clostridium acetobutylicum* to two important toxic metabolites: butanol and butyrate. Systems-level understanding is expected to lead to better strategies for industrial-strain development, as well as bioprocessing strategies taking advantage of the stress response to achieve superior bioprocessing outcomes.

Solventogenic and other clostridia are of major importance for developing technologies for biofuel production [1]. A major and unique advantage is their ability to utilize a large variety of substrates (hexoses, pentoses, oligosaccharides, xylan, and starches). Among the two sequenced solventogenic clostridia, *C. acetobutylicum* (*Cac*) is the only one that contains a full cellulosome [2] and may thus directly utilize cellulosic material for production of fuels and chemicals.

The toxic-metabolite stress response is a problem of major and general importance not only in clostridial biotechnologies but in all microbial systems of interest to bioenergy production [3]. In this project we investigated the metabolite stress response by collecting extensive transcriptomic (based on both deep sequencing and microarray analyses) and targeted fluxomic and proteomic data. These data have been used to identify differentially expressed genes during stress conditions along with the coupling of omics data integration with building stress models and modeling platforms that can be linked, as an added modeling dimension, to a 2nd generation GSM of this organism resulting in an in-depth systems-level molecular understanding at multiple genome-scale levels of the metabolite stress response.

Using RNA deep sequencing, we identified the role and expression of small non-coding RNAs (sRNAs) in stress response, apart from identifying 56 novel stress related sRNAs [4]. Furthermore, we identified the transcription factors and genetic circuits orchestrating the complex and multilayered response to butanol and butyrate stress [5]. Quantitative proteomic analysis was performed using iTRAQ tags. 566 and 588 unique proteins were identified and quantified from *Cac* grown under butanol and butyrate stress, respectively. 13C-Metabolic flux analysis is a widely used technique for measuring in vivo metabolic fluxes. Here, we have applied three 13C-labeled amino acid tracers, [1-13C]aspartate, [4-13C]aspartate, and [1-13C]serine to elucidate metabolic cycling between amino acid metabolism and central carbon metabolism in *Cac*. Such cycles are difficult to detect using traditional 13C-glucose tracers. We demonstrate, for the first time, that *Cac* has a highly active metabolic cycle between aspartate and

pyruvate. This metabolic cycle allows clostridium to rapidly interconvert several amino acids that are needed for cell growth and the adaptation to metabolite stress.

A genome-scale model, iCAC802, was developed for *Cac*, and includes 802 genes and 1470 reactions. The model was validated by comparison of in silico results to experimental gene deletion and 13C-MFA data. Gene transcription data for *Cac*'s response to butanol and butyrate stress was incorporated as regulation into the model using the E-Flux method. The regulated model exhibited reduction in biomass yield and down-regulation of glucose uptake as observed experimentally under stress conditions. The regulated model could be used for stress-specific metabolic predictions to aid redesign in pursue of a desired phenotype.

This multidimensional platforms and models based approach leads to an in-depth understanding of the metabolite stress response at molecular level to create a more streamlined genome and engineered strains with a better understanding of the complexity of network and regulation at molecular level. This aspect of project will further result in the development of computational and bioinformatics tools and frameworks towards modeling other complex cellular programs. Overall, this project's outcomes aim to become at enabling paradigm for modeling complex programs of organisms and biological systems of importance to DOE's mission on energy and the environment.

References

1. Tracy, B.P., et al., *Clostridia: the importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications.* Curr Opin Biotechnol, 2011.
2. Nolling, J., et al., *Genome sequence and comparative analysis of the solvent-producing bacterium Clostridium acetobutylicum.* J Bacteriol, 2001. **183**(16): p. 4823-38.
3. Nicolaou, S.A., S.M. Gaida, and E.T. Papoutsakis, *A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: From biofuels and chemicals, to biocatalysis and bioremediation.* Metab Eng, 2010. **12**(4): p. 307-31.
4. 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.
5. Wang, Q., et al., *Transcription factors and genetic circuits orchestrating the complex, multilayered response of Clostridium acetobutylicum to butanol and butyrate stress.* BMC Syst Biol, 2013. **7**(1): p. 120.

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