Metabolism in Microbial Communities and the Associated Biochemistry of Polymer Deconstruction

Authors: Janine Fu[1], Brendan Mahoney[1], John Muroski[1], Allen Takayesu[1], Orlando E. Martinez[1], Marco Morselli[1], Matteo Pellegrini[1], Rachel R. Ogorzalek Loo[1], Katherine Chou[2], Robert Clubb[1], Joseph A.. Loo[1] and Robert Gunsalus[1]* (robg@microbio.ucla.edu) and **Todd Yeates[1]**

Institutions: [1]UCLA DOE Institute, University of California, Los Angeles, Los Angeles, CA; [2] National Renewable Energy Laboratory, Bio Science Center, Golden, CO.

https://www.doe-mbi.ucla.edu/

Project Goals: Our microbiology team project within the UCLA DOE Institute employs a coordinated set of molecular and *in silico* approaches to examine model microbial communities and their component parts to better understand the processes that drive anaerobic carbon recycling in nature. These processes impact multiple areas of BER interest including bio-conversions of model substrates in natural and manmade environments, the associated biochemistry of key degradative enzymes and in the design of plant-based biomass deconstruction strategies for biofuel production. We are elucidating the undocumented metabolic pathways in syntrophic communities for model substrates with focus on key enzymes and associated oxidation reactions. We are developing next-gen omics methods to interrogate environmentally relevant pathways and interactions among organisms within microbial communities, and testing these proposed functions where possible. Using the model cellulolytic microorganism, *Clostridium thermocellum*, we are examining how anaerobic microbes synthesize and assemble their extracellular cellulosome structures that degrade lignocellulose.

Abstract text: Major activities within the UCLA-DOE Institute in the past year deal with three core areas of investigation.

Elucidation of syntrophic microbial pathways for metabolism of model substrates. Genomic, proteomic and informatic studies were performed on defined microbial communities to elucidate how representative fatty acid substrates are metabolized syntrophically. Key pathway enzymes were identified and characterized in two strains, *Syntrophomonas wolfei* and *S. wolfei* sub sp methylbutyratica for short and branched chain fatty acids along with the supporting electron transport reactions. Recombinant and structural studies of ten additional enzymes of the carbon oxidation pathway in *S. wolfei* were performed to further explore the biochemical basis for the thermodynamic limiting steps occurring during syntrophic cell growth. The resulting structures were also employed for subsequent modeling of acyl-lysine modifications of syntrophic pathway enzymes.

In companion studies, PacBio long read sequencing approaches are being used to sequence, assemble and annotate genomes of five previously unstudied syntrophic bacterial strains that utilize other model substrates when grown in co-culture with suitable methanogen partners. We are extending the gene annotation methods beyond the standard homology-based interferences to those based on co-evolution such as phylogenetic profiling, phenotypic profiling and operon conservation with the goal of supporting microbial pathway prediction and modeling.

Acyl-lysine modification of syntrophic pathway proteins. Proteomic and mass spectrometry studies were performed to further characterize protein post-translational modifications of carbon and electron transfer pathway enzymes in our model syntrophic strains. As protein modification can affect enzyme activity, these data will decipher their relationship with the metabolism of syntrophic microbial communities. Acyl-lysine modifications, which can arise from reactive metabolites, were strikingly found in high abundance in the proteome of model syntrophic bacteria. In *S. aciditrophicus*, *S. wolfei*, and *S. wolfei* sub sp *methylbutyratica*, multiple types of acyl-modifications were identified, including three types not reported before in any other organism. We have also identified the proteins modified and their sites of modification. Our recent work has shown that the type and relative abundance of these modifications do significantly change in response to different carbon sources, correlating with metabolic bottleneck points in the microbes' degradation pathway. These findings give us a glimpse at how thermodynamically-challenged organisms employ reversible catalysis to survive.

Cellulosome assembly and display in cellulolytic anaerobic bacteria. In companion microbial studies we are investigating how highly cellulolytic anaerobic bacteria synthesize, assemble and display cellulosomes. *Clostridium thermocellum,* a model bacterium capable of directly converting cellulosic substrates into ethanol and other biofuels is being used to investigate how the cell fine-tunes the enzyme composition of its cellulosome using anti-σ factors to control gene expression in response to sensing extracellular polymers. Our recent studies have shown that the RsgI9 anti-σ factor interacts with cellulose via a C-terminal bi-domain unit. A 2.0 Å crystal structure reveals that the unit is constructed from S1C peptidase and NTF2-like protein domains that contain a potential binding site for cellulose. Small angle X-ray scattering experiments of the intact ectodomain indicate that it adopts a bi-lobed, elongated conformation. In the structure a Conserved RsgI Extracellular (CRE) domain is connected to the bi-domain via a proline-rich linker, which is expected to project the carbohydrate binding unit ~160 Å from the cell surface. The CRE and proline-rich elements are conserved in several other *C. thermocellum* anti-σ factors, suggesting that they will also form extended structures that sense carbohydrates. We hypothesize that cellulolytic anaerobic bacteria assemble and display cellulosomes using a conserved molecular pathway. We are employing *in silico* comparative genomics approaches to identify conserved pathway components whose functional importance is being assessed in *C. thermocellum*. The results of these studies will provide new insight into anaerobic carbon recycling by naturally cellulolytic bacteria and could guide rational engineering efforts to create microbes that are capable of converting of plant biomass into biofuels, materials and chemicals.

References/Publications:

Muroski JM, Fu JY, Nguyen HH, Ogorzalek Loo RR, and Loo JA. Leveraging immonium ions for targeting acyl-lysine modifications in proteomic datasets. Proteomics. 2021 Feb;21(3-4).

Muroski JM, Fu JY, Nguyen HH, Wofford NQ, Mouttaki H, James KL, McInerney MJ, Gunsalus RP, Loo JA, Ogorzalek Loo RR. The acyl-proteome of *Syntrophus aciditrophicus* reveals metabolic relationship with benzoate degradation. (in revision).

Mahoney BJ, Takayesu A, Zhou A, Cascio D, Clubb RT. The structure of the *Clostridium thermocellum* RsgI9 ectodomain provides insight into the mechanism of biomass sensing. (submitted).

Funding statement: *This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-FC-02-02ER63421.*