Title: Metabolism in Microbial Communities and the Associated Biochemistry of Polymer Deconstruction

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Project Goals:

Our UCLA DOE Institute microbiology project area goals are to employ a coordinated set of molecular and *in silico* approaches to examine model microbial communities and their component parts to better understand the complex interactions and 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. With other UCLA DOE Institute members, we are initiating development of next-gen omics methods to interrogate environmental and genomic interactions between multiple organisms within a microbial community, and where possible, test these proposed functions.

Abstract text:

Major activities within the UCLA-DOE Institute in the past year deal with the investigation microbial metabolic processes central to global carbon cycling and biofuel production. We are employing molecular and *in silico*-based approaches to analyze anaerobic plant biomass degradation pathways in model syntrophic and fermentation-based microbial systems.

Abstract text:

Elucidation of syntrophic microbial pathways for metabolism of model substrates.

Proteomic and informatic studies were performed on defined microbial communities to elucidate how model environmental fatty acid substrates are metabolized into their gaseous end products. All key pathway enzymes were identified in the syntrophic bacterium, *Syntrophomonas wolfei* for C4-C8 chain length oxidations along with the supporting electron transport reactions to generate hydrogen gas and acetate. Analogous proteomic co-culture studies with the *Methanospirillum hungatei* community partner reveal a conserved carbon dioxide reduction pathway reliant on a core hydrogenase and formate dehydrogenase. Recombinant studies of five key enzymes of the carbon oxidation pathway in *Syntrophomonas wolfei* were performed by cloning, expression and purification to better define the biochemical basis for the thermodynamic limiting steps occurring during syntrophic cell growth. Protein structures have also been generated for two of these core syntrophic pathway enzymes for subsequent modeling.

Acyl-lysine modification of syntrophic pathway proteins.

Proteomic and mass spectrometry methods were also performed to characterize protein post-translational modifications of the above carbon and electron transfer pathways to decipher their relationship with the metabolism of syntrophic microbial communities. Acyl-lysine modifications affecting protein function are among the most striking findings from the proteome analysis of the model syntrophic bacteria. The abundance of such acylations in *Syntrophus aciditrophicus* and *Syntrophomonas wolfei* is sufficient to circumvent the pan-specific antibody enrichment steps (and their biases) typically employed in acylation studies. By analyzing the identity of the modified proteins, the sites of modification, and the composition of the modifications, we glimpse at how thermodynamically-challenged organisms can employ reversible catalysis to survive, balancing chemical degradation, synthesis, and excess reducing equivalents. Another feature of our studies is the development of mass spectrometry methods based on immonium ions that confidently identify acyl modifications in proteomes.

Cellulosome assembly and display in cellulolytic anaerobic bacteria.

In complementary microbial studies we are investigating how highly cellulolytic anaerobic bacteria 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 that control gene expression by sensing extracellular polymers. Here we present our progress toward understanding the function of RsgI9, a novel anti-σ factor whose function has yet to be determined. Using a combination of NMR spectroscopy, X-ray crystallography and biochemical assays, we have discovered that RsgI9's ectodomain comprises S1C protease-like and NTF2-like domains that cooperatively bind to cellulose. Here we present the atomic structures of RsgI9's ectodomain and solution-state characterization using small-angle X-ray scattering (SAXS), and our ongoing work to define RsgI9 regulates cellulase gene transcription. We hypothesize that cellulolytic anaerobic bacteria assemble and display cellulosomes using a conserved molecular pathway. We are therefore 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.

Our activities interface with other UCLA DOE Institute technical and plant research groups to complement research activities here and at the DOE National Laboratories and Bioenergy Research Centers in translational science applications.

References/Publications:

1. Muroski JM, Fu JY, Nguyen HH, Ogorzalek Loo RR, and Loo JA. “Leveraging immonium ions for targeting acyl-lysine modifications in proteomic datasets.” Proteomics, in press. (https://doi.org/10.1002/pmic.202000111)

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