Microbial Metabolism, Chemistry, and Communities under Study at the UCLA-DOE Institute for Genomics and Proteomics

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Project Goals: Research in the UCLA-DOE Institute for Genomics and Proteomics includes major efforts to elucidate critical microbial processes that decompose and recycle plant, animal and microbial biomass. Towards this end, we seek to decipher the metabolism of syntrophic microbial communities and examine how anaerobic microbes assemble complex cellulosome structures that degrade lignocellulose.

Biomass decomposition and recycling occur in essentially all anaerobic habitats on Earth, as well as in industrial/municipal waste treatment applications. Unfortunately, the current understanding of these critical processes is insufficient to enable modeling and prediction of environmental carbon flow. The benefits from increasing our knowledge of anaerobic decomposition include optimizing the attack and release of plant wall-derived molecules destined for biofuel and industrial feedstock production and improving biogas/sewage and waste stream processing plant design and operation.

Our DOE sponsored research seeks to advance the understanding of syntrophic-based microbial metabolism at molecular- and systems-levels and its role in biomass recycling/remediation. Exploring the pathways and key enzyme reactions of syntrophy begins by mining the genomes of previously unstudied syntrophic bacteria, such as those that metabolize model aliphatic fatty acid and amino acid substrates. That only minimal experimental data pertaining to these organisms is available severely limits the ability to draw conclusions from genome sequence alone, and even adding transcriptomic data may not suffice. For example, *Syntrophomonas wolfei* subspecies methylbutyratica possesses 9-11 paralogs for each reaction in the proposed beta-oxidation pathway along with distinct proteins for metabolizing branched chain fatty acids. Hence, quantitative proteomics is an important contributor, exposing which paralogue levels increase/decrease in response to substrate availability.

Unique to the UCLA efforts are the additional insights reaped by mining the proteomic mass spectrometry data, including how the pathways and key enzyme reactions of syntrophy may be modulated by post-translational modifications, an especially important consideration given that certain reactions possess equilibrium constants near 1. For example, fatty acid acylation of lysines in *Syntrophomonas wolfei* and *Syntrophus aciditrophicus* reflect substrate and metabolic intermediate levels.

In a second research project we are studying *Clostridium thermocellum,* which exhibits the highest level of cellulolytic activity of any microbe thus far characterized and is actively being developed for use in the consolidated bioprocessing of plant biomass into biofuels and chemicals. Its impressive ability to degrade lignocellulose is derived from the activity of a huge surface displayed enzyme complex called a cellulosome, which coordinates the binding of an array of cellulases. Only a few bacterial species within

the order Clostridiales are known to have evolved the capacity to display cellulosomes. Understanding how these microbes display these complex structures is of fundamental interest and could facilitate the construction of recombinant cellulolytic bacteria that have useful industrial applications. We are using an interdisciplinary approach to identify key components of the cellulosome biogenesis machinery. The aims project are to 1) Identify core components of the protein export machinery that produces the cellulosome. 2) Discover secretion stress and quality control systems involved in cellulosome biogenesis. 3) Elucidate the molecular basis of cellulosome tethering to the cell wall. Here we present our recent research progress, with particular focus on our studies RsgI-9, a novel member of a group of clostridial anti-sigma factors that regulate gene transcription of specific cellulosome components by sensing extracellular polysaccharide biomass components.

This work was funded by the Department of Energy BER Office of Science award # DE-FC02-02ER63421.