Unraveling New Mechanisms for Lignin Catabolism in Nature

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

A primary CBI goal is to understand how lignin is converted by microbes to facilitate cost-effective, efficient lignin valorization. *Pseudomonas putida* KT2440 is a Gram-negative soil bacterium reported to efficiently catabolize a variety of aromatic compounds. We have also demonstrated that *P. putida* can utilize high molecular weight lignin [1]. The intracellular mechanisms implicated in the catabolism of aromatic compounds have been extensively studied, but the enzymes involved in the breakdown of oligomeric lignin and/or their spatial location remains unknown. The work presented here provides new insights into the location of aromatic-catabolic and ligninolytic enzymes during microbial conversion of lignin.

To identify the enzymes involved in lignin breakdown, we performed a differential proteomics study in the intracellular and extracellular fraction of *P. putida* when grown in lignin and minimal media. The number of proteins found exclusively in lignin was considerably higher in the extracellular fraction than in the intracellular one and the former fraction contained oxidoreductases and aromatic-catabolic enzymes that had been previously described to be intracellular. To discern between cell lysis and secretion of enzymes to the extracellular milieu, we conducted cytometry and microscopy analysis. Cytometry revealed that 5 and 12% of the population were dead cells in minimal and lignin media, respectively. However, scanning and transmission electron microscopy images also uncovered the presence of outer membrane vesicles (OMVs), both in lignin and minimal media.

With the aim of understanding the function of these vesicles, we first analyzed their cargo through proteomics analysis. For that purpose, we isolated the vesicles from *P. putida* cultures grown in minimal media and lignin. The results indicate that some proteins were enriched in the

OMV fraction compared to the supernatant. In fact, some of the enzymes enriched in OMVs from lignin cultures have been previously reported to be involved in the catabolism of aromatic compounds or correlated with lignin breakdown in other organisms. This discovery opens new directions for investigation, from fundamental research to biotechnological applications, to understand how bacteria interact with lignin or aromatic compounds in the extracellular locus and to engineer improved microbes for the conversion of lignin to renewable chemicals.

References

1. Salvachúa, D., Karp, EM, Vardon, D.R., Nimlos, C., Beckham, G.T.*, 2015. Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria. Green Chemistry, 17, 4951-4967.

The Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.