BIOCONVERSION OF LEVULNIC ACID TO METHYL-ETHYL KETONE VIA A NOVEL CATABOLIC PATHWAY

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Key Words: Bioprocessing, metabolic engineering, lignocellulose, levulinic acid, *Escherichia coli*.

Levulinic acid (LA) is a common degradation by-product of acid-catalyzed hydrolysis of lignocellulosic biomass. Under the right conditions, LA can instead be made as the dominant hydrolysis product. For this reason, LA was identified as a key renewable building block and motivated development of chemical upgrading strategies for producing value-added chemicals from a renewable feedstock. Biological upgrading of LA has been limited to natural microbial products, mainly polyhydroxyalkanoates (PHA), due to a lack of understanding about the LA catabolic pathway. Recently, we discovered and characterized an enzymatic pathway from *Pseudomonas putida* KT2440 that enables growth on LA in both *P. putida* and when heterologously expressed *Escherichia coli* LS5218. The pathway, described in Rand *et al.*, *Nature Microbiology* (2017), activates LA as a CoA-thioester, reduces 4-keto-moiety, and isomerizes the resulting hydroxyl via a unique ATP-dependent mechanism. The 3-hydroxypentanoyl-CoA generated by the pathway can be incorporated into PHA or further oxidized by the native enzymes of beta-oxidation to enable growth and energy generation. In this talk, we will describe the transposon (traditional and TN-seq) experiments used to identify essential genes in the pathway and the genetic and biochemical studies used to associate each enzyme in a five gene operon (*lvaABCDE*) to roles in the pathway.

We will also describe our efforts to evolve *E. coli* for utilization of LA as a sole carbon source. The evolved strains were derived from *E. coli* LS5218, which contains specific mutations for overexpression of β-oxidation (*fadR*) and short chain fatty acid degradation genes (*atoC(Con)*). Genome sequencing of the evolved mutants and parent led to the isolation of two key function deletions required for robust growth on LA as well as several other differences away from its presumed *E. coli* parent. Reconstitution of the isolated mutations in wild type LS5218 revealed one to be necessary, *fadE*, and one to confer a beneficial growth phenotype, *atoC*. In this talk, we will describe the genome of LS5218 (Rand *et al.*, *Metabolic Engineering Communications* (2017), and how it may be an advantageous strain for metabolic engineering of organic acid biosynthesis.

Lastly, we will describe how we engineered a strain of *E. coli* to perform a bioconversion of LA to the common industrial solvent methyl-ethyl ketone (MEK). Here, we overexpressed an acetoacetyl-CoA transferase (AtoDA) and heterologously expressed an acetoacetate decarboxylase (ADC) to enable flux to MEK. Through genome scale metabolic modeling of the pathway, we identified growth coupling strategies to force cells to perform the bioconversion in order to generate energy (and grow). The resulting strains convert LA to MEK at unit conversion with appreciable rates. In this talk, we will present the unpublished metabolic engineering, modeling, and fed-batch optimization of the process.