

## Genome shuffling and bacterial quantitative trait locus (QTL) mapping in *Pseudomonas putida*

Julie E. Chaves<sup>1\*</sup> ([chavesje@ornl.gov](mailto:chavesje@ornl.gov)), Delyana P. Vasileva<sup>1</sup>, Jared C. Streich<sup>1</sup>, Leah H. Burdick<sup>1</sup>, Dawn M. Klingeman<sup>1</sup>, Daniel A. Jacobson<sup>1</sup>, Joshua K. Michener<sup>1</sup>, and **Gerald A. Tuskan<sup>1</sup>**

<sup>1</sup>Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, TN

<https://cbi.ornl.gov>

**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 addresses 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, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

An ideal bacterium for industrial bioconversions would rapidly assimilate varied potential feedstocks, withstand extreme environmental conditions, and efficiently generate a useful product. Each of these desirable traits is highly polygenic, with multiple alleles making quantitative contributions to the overall phenotype. The pangenome of a bacterial species is expected to have more beneficial alleles than would be present in any individual isolate; for example, two strains evolve independent mechanisms (and alleles) that increase inhibitor tolerance. Therefore, identifying and recombining alleles between strains can improve performance.

*Pseudomonas putida* is an attractive host organism for the conversion of lignocellulose to specialty biofuels due to its natural ability to tolerate and metabolize lignin-derived aromatic monomers. We are optimizing methods for genome shuffling of *Pseudomonas putida* strains displaying desirable traits. For selectable phenotypes of interest, such as tolerance to inhibitory chemicals found in depolymerized substrates (e.g., biomass hydrolyzates), we can directly select improved progeny and identify the causal genetic changes. Additionally, measuring genotypes and phenotypes across a panel of shuffled progeny allows the mapping of genetic determinants for non-growth-associated phenotypes such as product formation. These genetic loci can then be used as targets for rapid future rational engineering efforts.

*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.*