Integrated *Pseudomonas putida* Strain Design For Maximizing Biomass Conversion To Biofuels And Bioproducts.

Deepanwita Banerjee[1,2*](dbanerjee@lbl.gov), Deepika Awasthi[1,2*](dawasthi@lbl.gov), Hyun Gyu Lim[1,3], Manuel Rafael Jimenez Diaz[1,2], Thomas Eng[1,2], Blake A. Simmons[1,2], Steven W. Singer[1,2], Adam Feist[1,3,4], Aindrila Mukhopadhyay[1,2] and **Jay D. Keasling**[1,2,4,5]

[1]Joint BioEnergy Institute, 5885 Hollis street, 4th floor, Emeryville, CA 94608, USA; [2]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94702, USA; [3]Department of Bioengineering, University of California San Diego, 9500 Gilman Dr., La Jolla, CA 92093, USA; [4]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark and [5]Center for Synthetic Biochemistry, Institute for Synthetic Biology, Shenzhen Institutes for Advanced Technologies, Shenzhen, China

http://jbei.org

Project Goals: Engineer novel catabolic routes to redirect carbon flow, use of functional genomics and predictive tools for systematic improvements in titer, rate, and yield (TRY) and use of adaptive laboratory evolution (ALE) to enhance host phenotype.

Plant biomass (lignocellulose) is an underutilized carbonaceous feedstock in the industrial microbial bioconversions. Though polymers of glucose, xylose and aromatic hydrocarbons form lignocellulose, only glucose bioconversion to products is the commercially established bioprocess. In the presented work we show an integrated host engineering strategy that can achieve maximum lignocellulosic carbon bioconversion to heterologous drop-in biofuels and bioproducts by engineering *Pseudomonas putida*, a non-pathogenic soil microbe. Our approach integrates A) targeted *P. putida* strain engineering to incorporate heterologous non-oxidative glycolysis to limit CO2 loss and reach theoretical maximum product yields from glucose, incorporate heterologous xylose catabolism capability and express a multi-gene heterologous pathway for methyl ketones (diesel substitute) production; B) adaptive laboratory evolution for improved growth on xylose and galactose as well as tolerance to lignin-derived aromatics and ionic liquids; and C) high throughput fitness profiling and genome-scale metabolic modeling (GSMM) to identify experimentally implementable gene deletion targets for enhanced productivity and fitness under bioreactor conditions. These host developments can help improve the titers, rates and yields (TRY) of bioproducts under industrial bioprocess parameters as modeled with a sustainable pigment, indigoidine, or in future studies with advanced biofuel candidates, methyl ketones and isopentenol.

Publications

1. Banerjee, D., Eng, T., Lau, A. K., Sasaki, Y., Wang, B., Chen, Y., … Mukhopadhyay, A. (2020). Genome-scale metabolic rewiring improves titers rates and yields of the non-native product indigoidine at scale. *Nature Communications*, *11*(1), 5385.
2. Lim, H. G., Fong, B., Alarcon, G., Magurudeniya, H. D., Eng, T., Szubin, R., … Feist, A. M. (2020). Generation of ionic liquid tolerant *Pseudomonas putida* KT2440 strains via adaptive laboratory evolution. *Green Chemistry*, 22(17), 5677–5690.

This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.