Isoprenol production in *P. putida* KT2440

Ian S. Yunus[1,2,*] (ianyunus@lbl.gov), Xi Wang[1,2], Deepanwita Banerjee[1,2], Joonhoon Kim[1,2], Yan Chen[1,2], Jennifer Gin[1,2], Chris J. Petzold[1,2], Paul D. Adams[1,3,5], Aindrila Mukhopadyay[1,2], **Taek Soon Lee**[1,2], Jay D. Keasling[1,2,5,6] (Project PI)

[1] Joint BioEnergy Institute (JBEI), 5885 Hollis St., Emeryville, CA 94608, USA
[2] Biological Systems & Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[3] Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[4] Department of Molecular & Cell Biology, University of California, Berkeley, CA 94720, USA
[5] Department of Bioengineering, University of California, Berkeley, CA 94720, USA.
[6] Department of Chemical & Biomolecular Engineering, University of California, Berkeley, CA 94720, USA.

www.jbei.org

Project Goals: Establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.

Isoprenol (3-methyl-3-buten-1-ol) is a candidate for a drop-in biofuel and a precursor for commodity chemicals (e.g., rubber), and more recently it was demonstrated as a precursor for a promising sustainable aviation fuel (SAF) compound DMCO (1,4-dimethylcyclooctane)[1,2]. Isoprenol has been produced in various engineered microorganisms, including *E. coli, C. glutamicum,* and *S. cerevisiae*. Recently, *Pseudomonas putida* KT2440 has gained interest as a promising host for bioproduction of drop-in biofuels as it can utilize carbon sources generated from inexpensive plant biomass. However, the potential of *P. putida* KT2440 for isoprenol production remained unexplored. In this study, we aim to engineer *P. putida* KT2440 for isoprenol production. First, we employed opt-approaches and constrained minimal cut sets (cMCS) to identify gene knockout targets to maximize isoprenol production. Secondly, we established an "IPP-bypass" isoprenol pathway by utilizing a promiscuous mevalonate diphosphate decarboxylase to alleviate the toxicity imposed by an intermediate metabolite (isopentenyl diphosphate, IPP). Next, we further improved the isoprenol titer by optimizing the pathway protein expression. Lastly, we employed targeted proteomics to identify the bottlenecks in the pathway. Altogether, the highest isoprenol production titer of 1.1 g/L was achieved from the engineered *P. putida* KT2440 strain.

Reference

[1]Kang, A. *et al. Metab. Eng.* 56, 85–96 (2019)
[2]Baral, N. R. *et al. ACS Sustain. Chem. Eng.* 9, 11872–11882 (2021)

Funding Statement: *Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.*