Isopentenol Production Using the IPP-Bypass Mevalonate Pathway

Daniel Mendez-Perez[1,2], Xi Wang[1,2], Aram Kang[1,2], Aindrila Mukhopadhyay[1,2], and

Taek Soon Lee[1,2,*] (tslee@lbl.gov)

[1]Joint BioEnergy Institute, 5885 Hollis Street, Emeryville, CA 94608, USA.
[2]Biological Systems & Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

http://www.jbei.org/research/divisions/biofuels-and-bioproducts/pathway-metabolic-engineering/

Project Goals: Isopentenol is a drop-in biofuel and a precursor for commodity chemicals, and biosynthetic pathways to this product have been developed. But most production study has been limited to batch experiments in shake flasks. In this study, we explored isopentenol production via IPP-bypass pathway in a 2-L bioreactor using fed-batch fermentation as an initial scale-up demonstration of the technology. With a success of the initial scale up effort, we may attempt the production of isopentenol at a commercially more relevant scale in the future.

Isopentenol (3-methyl-3-buten-1-ol, isoprenol) is a valuable compound as a drop-in biofuel and a precursor of commodity chemical such as isoprene. Synthetic microbial system using heterologous mevalonate pathway has been developed for the production of isopentenol via extensive metabolic engineering efforts[1]. However, the toxicity of key intermediate isopentenyl diphosphate (IPP) has been a significant bottleneck of high titer production of isopentenol in microbial hosts[2]. In previous study, we have developed an IPP-bypass pathway for isopentenol production which bypasses the toxic intermediate formation as well as relieves energy demand of the original mevalonate pathway[3,4]. This alternative pathway has been developed and further engineered for the production of isopentenol in *E. coli*. However, production studies have been limited to batch experiments in shake flasks. In this study we tested isopentenol production via IPP-bypass pathway in a 2-L bioreactor using fed-batch fermentation. After several optimization strategies such as media optimization and the elimination of the acetate generating pathways, isopentenol production reached 10.8 g/L, which is the highest reported titer for this compound. To achieve this high titer, we found that it is required to use a two-phase fermentation process in which isopentenol is partially removed from the culture media to organic phase (with oleyl alcohol) to relieve the toxic effects by isopentenol. This IPP-bypass pathway was also engineered in *P. putida* for initial production of isoprenoids.

References

1. George, et al., *Biotech. Bioeng.* **2014**, *111*, 1648-1658 (doi:10.1002/bit.25226)

2. George, et al., *Metabol. Eng*. **2018**, *47*, 60-72 (doi: 10.1016/j.ymben.2018.03.004)
3. Kang, et al., *Metabol. Eng*. **2016**, *34*, 25-35 (doi: 10.1016/j.ymben.2015.12.002)
4. Kang, et al., *Metabol. Eng*. **2017**, *41*, 125-134 (doi: 10.1016/j.ymben.2017.03.010)

Funding statement

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.