

130. Advanced pathways for microbial production of branched C5 alcohols

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Project Goals: The Joint BioEnergy Institute (JBEI) aims to produce a chemically diverse suite of biofuels from lignocellulosic biomass. Isoprenoid-based biofuels have been of great interest due to their superb fuel properties such as low freezing temperature and high octane number. Mevalonate (MVA) pathway is one of the major biosynthetic pathways of isoprenoid fuel production, and the engineering of this pathway is a key approach to achieve higher production of these biofuels. Various engineering strategies and tools have been explored to identify the bottlenecks of the pathway and to understand the pathway enzymes better, but the intrinsic energy demands of this pathway and the operational cost for aeration to meet the energy demands have been still a problem when a production in large scale using fermentor is exploited. In this work, we present modified version of the MVA pathway that will address these issues for isoprenoid biofuel production. Isoprenoids are considered as one of the most promising advanced biofuels. Among the isoprenoid compounds, branched five carbon (C5) alcohols have been tested as good biofuel compounds with favorable combustion properties and octane numbers to gasoline. A synthetic pathway for C5 alcohols production has been reported previously in *E. coli* using the heterologous MVA pathway (Chou and Keasling, 2012), and further metabolic engineering efforts on this synthetic pathway have led to about 50% theoretical yield in the C5 alcohol production (George et al. 2014). Even though the MVA pathway has been known to be less efficient than the methylerythritol phosphate (MEP) pathway in carbon and redox balance as well as in energy balance, the MVA pathway has been extensively used for microbial production of a range of valuable isoprenoids due to its tractability and the high titers it can provide. However, the energy demands of this pathway and the operational cost for aeration to meet these energy demands have been a problem, especially when a production in large scale using fermentor is exploited. In this work, we present modified pathways for C5 alcohol production that will address these issues of the traditional MVA pathways. We engineered advanced pathways for C5 alcohol production that reduce cellular costs for isopentenol production. One of the modified pathways showed that isopentenol could be produced via decarboxylation of mevalonate monophosphate to isopentenyl monophosphate by the promiscuous activity of the decarboxylase. The titer and the growth of the engineered strains with this modified pathway were better than those with the original pathway, and currently the efficiency of this modified pathway is tested under microaerobic condition. The decarboxylase enzyme engineering and the pathway optimization of this modified pathway would lead a microbial C5 alcohol production more economically feasible, especially for large scale industrial application.

References

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