229. Engineering a Balanced MEV Pathway for Biofuels Production

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Project Goals

The mevalonate pathway (MEV) has the potential to produce biofuels such as limonene and bisabolene. The heterologous expression of MEV in *E. coli* removes undesired regulatory points and enables higher titers of the target terpene. However, the unregulated over-expression of enzymes leads to an accumulation of metabolites with toxic and inhibitory effects. Therefore, it is essential to coordinate the relative levels of the pathway enzymes to improve production titers. The goal of this project is to develop tools for pathway balance, and therefore, to achieve high titer production of biofuel compounds.

Abstract

In the present study we showed two different approaches for balancing heterologous MEV gene expression in E.coli and improve production from 1% glucose: i) Engineer a dynamic regulation of the pathway using promoters responding to an accumulation of the toxic intermediate FPP,[1] which improved bisabolene titer to 875 mg/L ii) Explore expression of the MEV pathway enzymes using targeted proteomics and its associated limonene production[2] to define engineering strategies; a principal component analysis (PCA) of the proteomics and the production at various conditions suggests that an over-expression of the bottom portion of the pathway and a moderate expression of the top portion are keys to improve the production. Indeed, extra copies of the terpene synthase and a modified top portion led to 665 mg/L and 1150 mg/L of limonene and bisabolene productions, respectively. This represents a 40% increase in production titer over previous, unbalanced systems using the same genes2.

References

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