

Title: Isopentenyl diphosphate (IPP)-bypass mevalonate pathways for C₅ alcohol production

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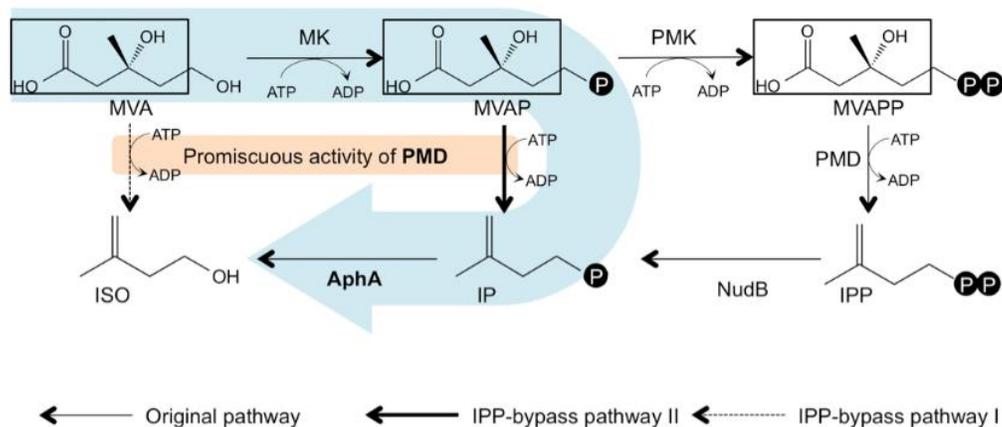
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Project Goals: The Joint BioEnergy Institute (JBEI) aims to produce a chemically diverse suite of biofuels from lignocellulosic biomass, and isoprenoid-based biofuels have been of great interest due to their superb fuel properties. Mevalonate (MVA) pathway is one of the major biosynthetic pathways of isoprenoid fuel production. Various engineering strategies and tools for this pathway have been explored to identify the bottlenecks of the pathway and to achieve higher production of these biofuels. The toxicity of key intermediates and the intrinsic energy demands of this pathway, which result a significant operational cost for aeration, have been two important problems when a production in large scale is exploited. In this work, we present modified version of the MVA pathway that will address these issues for isoprenoid biofuel production, and our recent efforts to improve isopentenol production using this modified pathway.

Isopentenol (3-methyl-3-butenol) is a promising biofuel with favorable combustion properties and a precursor molecule for the production of isoprene (1). A mevalonate (MVA)-based isoprenoid biosynthetic pathway for C₅ alcohols was constructed in *E. coli* using genes from several organisms, and the pathway was optimized to achieve over 50% theoretical yield (2, 3). The MVA and MEP pathways intersect at isopentenyl diphosphate (IPP), the direct precursor to isoprenoid-derived C₅ alcohols and initial precursor to longer chain terpenes, which makes independent regulation of the pathways difficult.

In pursuit of the “decoupling” of the MVA pathway from native cellular regulation, we designed novel IPP-bypass MVA pathways for C₅ alcohol production by utilizing promiscuous activities of two enzymes, phosphomevalonate decarboxylase (PMD) and an *E. coli*-endogenous phosphatase (4).

The IPP-bypass pathways have reduced energetic requirements, are further decoupled from intrinsic regulation, and are free from IPP-related toxicity. In addition to these benefits, we demonstrate that reduced aeration rate has less impact on this bypass pathway than the original MVA pathway. Finally, we showed that performance of the bypass pathway was primarily determined by the activity of PMD. We designed a growth-linked screening platform to select PMD mutants with improved activity and demonstrated titer increases in the mutant strains. This modified pathway would be a good platform for industrial production of isopentenol and isoprene.



References

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