

Metabolic engineering of Mevalonate pathway in E. coli for isoprenoid fuel production

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Project Goal:

Mevalonate 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. Targeted proteomics and enzyme kinetics provide important information to achieve this goal, and a new design strategy using synthetic biology allows the combinatorial approach to find the best combination of the pathway genes for biofuel production. In this study, we present several engineering strategies of top portion and bottom portion of mevalonate pathway.

Mevalonate pathway has been explored and engineered as an important biosynthetic pathway for the production of isoprenoids in both E. coli and yeast. The engineering of mevalonate pathway to produce more IPP (isopentenyl diphosphate) and DMAPP (3, 3-dimethylallyl diphosphate) is a major approach to improve isoprenoid based fuel production. Targeted proteomics provides highly selective protein identification and inexpensive quantification of individual pathway proteins, and this tool can suggest bottlenecks of the metabolic pathway. The first bottleneck identified is HMG-CoA reductase (HMGR), which is one of the key enzymes in mevalonate pathway. By replacing the original NADPH-dependent HMGR into an NADH-dependent HMGR identified in the analysis of public genome database, we have improved the production about 50% higher and further improvement has been also achieved by increasing the intracellular NADH pool using formate dehydrogenase from Candida. Another bottleneck of the pathway is mevalonate kinase (MK) and phosphomevalonate kinase (PMK), and the kinetic study of these enzymes has been performed to understand the property of these enzymes better. Finally, based on the production and proteomics data we have acquired so far, we re-designed a large number of combinations of 8 separate genes in the mevalonate pathway under 3 operons in the same plasmid, which is pretty different from the original gene order in the operon and genetic context (or a relative position of individual gene within the pathway). We have prepared this combinatorial library of mevalonate pathway using newly developed BioCAD tool, j5, and robotics cloning tool. Using targeted proteomics and production profile, we can quantify mevalonate-pathway proteins for each variant to determine the effects of gene order on protein expression and actual biofuel production.