High-throughput enzyme screening platform for the IPP-bypass mevalonate pathway for isopentenol production

Isopentenol (or isoprenol, 3-methyl-3-buten-1-ol) is a drop-in biofuel and a precursor for commodity chemicals such as isoprene. Biological production of isopentenol via the mevalonate pathway has been optimized extensively in Escherichia coli, yielding 70% of its theoretical maximum. However, high ATP requirements and isopentenyl diphosphate (IPP) toxicity pose immediate challenges for engineering bacterial strains to overproduce commodities utilizing IPP as an intermediate. To overcome these limitations, we developed an IPP-bypass isopentenol pathway using the promiscuous activity of a mevalonate diphosphate decarboxylase (PMD) and demonstrated improved performance under aeration-limited conditions. However, relatively low activity of PMD toward the non-native substrate (mevalonate monophosphate, MVAP) was shown to limit flux through this new pathway. By inhibiting all IPP production from the endogenous non-mevalonate pathway, we developed a high-throughput screening platform that correlated promiscuous PMD activity toward MVAP with cellular growth. Successful identification of mutants that altered PMD activity demonstrated the sensitivity and specificity of the screening platform. Strains with evolved PMD mutants and the novel IPP-bypass pathway increased titers up to 2.4-fold. Further enzymatic characterization of the evolved PMD variants suggested that higher isopentenol titers could be achieved either by altering residues directly interacting with substrate and cofactor or by altering residues on nearby α-helices. These altered residues could facilitate the production of isopentenol by tuning either kcat or Ki of PMD for the non-native substrate. The synergistic modification made on PMD for the IPP-bypass mevalonate pathway is expected to significantly facilitate the industrial scale production of isopentenol.