High-throughput Screening Device Based Evolutionary Protein Engineering for Enhancing Itaconate Production from Acetate

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Utilizing acetate as a carbon source is a promising approach for sustainable development, as it is obtainable from abundant sources.
Itaconate is produced by cis-aconitate decarboxylase (cad) in one step reaction. However, there are hurdles when expressing cad in E. coli, as it has low activity when heterologously expressed and the pool of substrate, cis-aconitate, is low as it is the intermediate of TCA cycle. In this study, we leveraged evolutionary protein engineering based on recently identified structure of Cad for improving its properties and itaconate high-throughput screening sensor (HTS) for screening the Cad variants with better performances. Thereafter, curing of itaconate HTS sensor were conducted for validation of the screened Cad variants and found out that the screened mutants VR and GT showed 1.28-fold increase in itaconate titer. The results of enzyme assay demonstrated that the mutants VR and GT had 1.25-fold higher catalytic efficiency compared to wild type Cad, even though affinity was slightly lower (Km value 1.42-fold increase). The mutants will be additionally validated in final production strain to maximize the itaconate production.