

TCA flux hijacker by evolutionary protein engineering for efficient itaconate production in *Escherichia coli*

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Microbial production of itaconate has been mainly carried out by *Aspergillus terreus*. Recently, its production in *Escherichia coli* has been studied due to the complicated fermentation process and shortage of genetic engineering tools of *A. terreus*. One of the major issues on itaconate production in industrial strains is the poor availability of its precursor, *cis*-aconitate, unlike spatially compartmentalized itaconate synthesis of *A. terreus*. In this study, directed evolution of protein known to have substrate promiscuity to citrate was conducted in *E. coli* to enhance the *cis*-aconitate availability. Initially, the itaconate responsive screening system could be constructed by using the itaconate-responsive transcription factor (ItcR) from *Yersinia pseudotuberculosis*. Thereafter, the mutant library was rationally designed and enriched. Finally, the developed mutant showed significantly enhanced catalytic efficiency to citrate compared to the wild-type enzyme and consequently, its use enabled 4.26-fold increased itaconate production compared to the parental strain, indicating the successful enhancement of *cis*-aconitate supply as a hijacker of TCA flux.