

Metabolic engineering for the enhanced production of succinic acid based on genome comparison and *in silico* analysis in *Escherichia coli*

Tae Yong Kim¹, Sang Jun Lee^{1,2}, Dong-Yup Lee^{1,3}, and Sang Yup Lee^{1,2,3*}

¹Metabolic and Biomolecular Engineering National Research Laboratory,
Department of Chemical and Biomolecular Engineering,

²Center for Ultramicrochemical Process Systems,

³Department of BioSystems, BioProcess Engineering Research Center and
Bioinformatics Research Center, Korea Advanced Institute of Science and
Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Korea.

Two genomes of mixed-acid fermenting *Escherichia coli* and succinic acid over-producing *Mannheimia succiniciproducens* were compared to sort out candidate genes to be manipulated for overproducing succinic acid in *Escherichia coli*. This resulted in the identification of five genes or operons, including *ptsG*, *pykF*, *sdhA*, *mgo*, *aceBA*, which may drive metabolic flux away from succinic acid in the central metabolic pathway of *Escherichia coli*. By *In silico* analysis based on stoichiometric metabolic balance equations, correlation between maximum biomass and succinic acid production was obtained for combination of gene knockouts. Based on this *in silico* analysis and the comparison of genome, simultaneous disruption of three pyruvate forming enzymes, *ptsG*, *pykF* and *pykA* were found to affect fermentative metabolism of the cell profoundly. This triple mutation increased succinic acid ratio to fermentation products 9-fold and succinic acid production 5-fold.

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