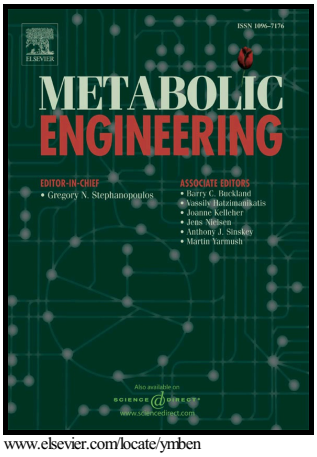

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PII: S1096-7176(16)30088-X
DOI: http://dx.doi.org/10.1016/j.ymben.2016.08.005
Reference: YMBEN1145

To appear in: *Metabolic Engineering*

Received date: 16 June 2016
Revised date: 16 August 2016
Accepted date: 18 August 2016

Cite this article as: Takahisa Kogure, Takeshi Kubota, Masako Suda, Kazum Hiraga and Masayuki Inui, Metabolic engineering of *Corynebacteriur glutamicum* for shikimate overproduction by growth-arrested cell reactior *Metabolic Engineering,* http://dx.doi.org/10.1016/j.ymben.2016.08.005

Metabolic engineering of Corynebacterium glutamicum for shikimate overproduction by growth-arrested cell reaction

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Abstract

Corynebacterium glutamicum with the ability to simultaneously utilize glucose/pentose mixed sugars was metabolically engineered to overproduce shikimate, a valuable hydroaromatic compound used as a starting material for the synthesis of the anti-influenza drug oseltamivir. To achieve this, the shikimate kinase and other potential metabolic activities for the consumption of shikimate and its precursor dehydroshikimate were inactivated. Carbon flux toward shikimate synthesis was enhanced by overexpression of genes for the shikimate pathway and the non-oxidative pentose phosphate pathway. Subsequently, to improve the availability of the key aromatics precursor phosphoenolpyruvate (PEP) toward shikimate synthesis, the PEP:sugar phosphotransferase system (PTS) was inactivated and an endogenous myo-inositol transporter IolT1 and glucokinases were overexpressed. Unexpectedly, the resultant non-PTS strain accumulated 1,3-dihydroxyacetone (DHA) and glycerol as major byproducts. This observation and metabolome analysis identified glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-catalyzed reaction as a limiting step in glycolysis. Consistently, overexpression of GAPDH significantly stimulated both glucose consumption and shikimate production. Blockage of the DHA synthesis further improved shikimate yield. We applied an aerobic, growth-arrested and high-density cell reaction to the shikimate production by the resulting strain and notably achieved the highest shikimate titer (141 g/l) and a yield (51% (mol/mol)) from glucose reported