Genome engineering of *Corynebacterium glutamicum* as a chassis for bioconversion of C1 feedstocks

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The present biomanufacturing process is highly depended on sugar-based resources. Recently, abundant natural gas supplies and feasible conversion of methane to methanol have made methanol a promising C1 feedstock with low cost and good availability for biomanufacturing. *Corynebacterium glutamicum* is a well-known industrial workhorse for amino acid production. Herein, facile yet robust genome editing tools were developed and applied for engineering *C. glutamicum* to serve as a promising chassis for bioconversion of C1 feedstocks.

To achieve efficient genome engineering of *C. glutamicum*, we developed a complex CRISPR/Cas9 based toolbox. Cas9 and gRNA expression cassettes were reconstituted to combat Cas9 toxicity and facilitate effective termination of gRNA transcription. CRISPR/Cas9-mediated gene deletion/insertion/replacement was achieved efficiently. Furthermore, Cas9 variants recognizing various PAMs and nucleotide (cytidine and adenine) deaminases were integrated into the toolbox and facilitated precise base editing and multiple-locus editing. The developed genome editing tools were then adapted to work with our robot-based integrative laboratory automation system, establishing a powerful BioFoundry platform. With this platform, we rationally designed and experimentally engineered *C. glutamicum* to serve as a promising chassis for methanol bioconversion. Specifically, pentose and methanol utilization pathways were reconstructed to engineer *C. glutamicum* as a methanol-dependent synthetic methylotroph. Its cell growth relies on co-utilization of methanol and xylose, and most notably methanol is an indispensable carbon source. Due to the methanol-dependent characteristic, adaptive laboratory evolution was successfully applied to improving methanol utilization. The evolved mutant showed a 40-fold increase in cell growth and utilized ^{13}C-methanol and xylose with a high mole ratio of 7:1 and ^{13}C-labeling of intracellular metabolites up to 63%. Methanol-dependent amino acid production was also achieved, demonstrating the potential in bioconversion of methanol into valuable chemicals. Genome resequencing combined with reverse metabolic engineering revealed key genetic factors involved in methanol utilization in *C. glutamicum*.

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Research Interests:

Synthetic Biology	Metabolic Engineering
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Selected publications

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