Controlling Citrate Synthase Expression by CRISPR/Cas9 Genome Editing for n-Butanol Production in Escherichia coli - DTU Orbit (09/11/2017)

Controlling Citrate Synthase Expression by CRISPR/Cas9 Genome Editing for n-Butanol Production in Escherichia coli
Genome editing using CRISPR/Cas9 was successfully demonstrated in Esherichia coli to effectively produce n-butanol in a defined medium under microaerobic condition. The butanol synthetic pathway genes including those encoding oxygen-tolerant alcohol dehydrogenase were overexpressed in metabolically engineered *E. coli*, resulting in 0.82 g/L butanol production. To increase butanol production, carbon flux from acetyl-CoA to citric acid cycle should be redirected to acetoacetyl-CoA. For this purpose, the 5′-untranslated region sequence of *gltA* encoding citrate synthase was designed using an expression prediction program, UTR designer, and modified using the CRISPR/Cas9 genome editing method to reduce its expression level. *E. coli* strains with decreased citrate synthase expression produced more butanol and the citrate synthase activity was correlated with butanol production. These results demonstrate that redistributing carbon flux using genome editing is an efficient engineering tool for metabolite overproduction.

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