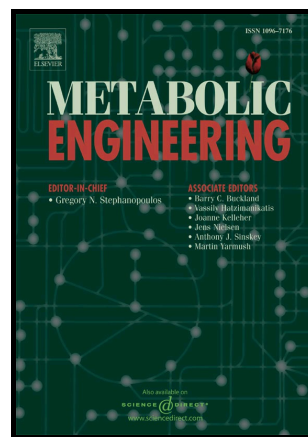


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Rediverting carbon flux in *Clostridium ljungdahlii* using CRISPR

Interference (CRISPRi)

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Abstract

Clostridium ljungdahlii has emerged as an attractive candidate for the bioconversion of synthesis gas (CO, CO₂, H₂) to a variety of fuels and chemicals through the Wood-Ljungdahl pathway. However, metabolic engineering and pathway elucidation in this microbe is limited by the lack of genetic tools to downregulate target genes. To overcome this obstacle, here we developed an inducible CRISPR interference (CRISPRi) system for *C. ljungdahlii* that enables efficient (>94%) transcriptional repression of several target genes, both individually and in tandem. We then applied CRISPRi in a strain engineered for 3-hydroxybutyrate (3HB) production to examine targets for increasing carbon flux toward the desired product. Downregulating phosphotransacetylase (*pta*) with a single sgRNA led to a 97% decrease in enzyme activity and a 2.3-fold increase in titer during heterotrophic growth. However, acetate production still accounted for 40% of the carbon flux. Repression of aldehyde:ferredoxin

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