Multiplexed transcriptional control strategies for biosynthesis from mixed substrates in *Escherichia coli*

by

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Technical summary

Metabolic engineering reprograms microbes to produce value-added chemicals. Microbial production has the potential to use renewable feedstocks, such as conventional waste streams. Metabolic engineers already contend with the metabolic burden of recombinant production pathways; utilizing complex input streams only further complicates allocating cellular resources appropriately for biosynthesis. This thesis aims to develop transcriptional control strategies that sense and respond to changing feedstock conditions for biosynthesis and demonstrate the ability to produce a product of interest from mixed substrate feeds.

We constructed a galacturonate biosensor with the galacturonate-responsive transcription factor, ExuR, from *Bacillus subtilis* and determined the best performer from a selection of biosensor variants. After establishing no interactions with the host *Escherichia coli* native regulatory system, we applied the biosensor to control expression of biosynthetic pathway. It was confirmed that the biosensor activated transcription in the presence of galacturonate, eliminating the need for a chemically-inducible control system.

A second, gluconate biosensor was constructed with GntR, from *B. subtilis*. The two biosensors were shown to be orthogonal and each was used to control the expression of a novel D-glycerate biosynthetic pathway from its cognate substrate. We demonstrated D-glycerate production from single and mixed substrate feeds and showed that mixed substrates in different ratios resulted in fairly consistent titers.

This work demonstrates the engineering of expression controllers and a production strain for biosynthesis from mixed substrate feeds.

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