Evolution-guided optimization of biosynthetic pathways

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Introduction and motivation

Biosynthetic chemical production has benefits over other synthesis methods.

Can cellular mechanisms be optimized to yield higher concentrations of desired chemicals?

- Mutated genome of *E. coli* cells and guided through evolution to yield high-producing strains
- Used flux balance analysis (FBA) to identify key genes in the production of naringenin and glucaric acid
- Evaluated success by comparing chemical production titers against those of unmodified cells

Experiment significance

Harnessing evolution to optimize metabolic pathways

Efficient, high-throughput chemical production

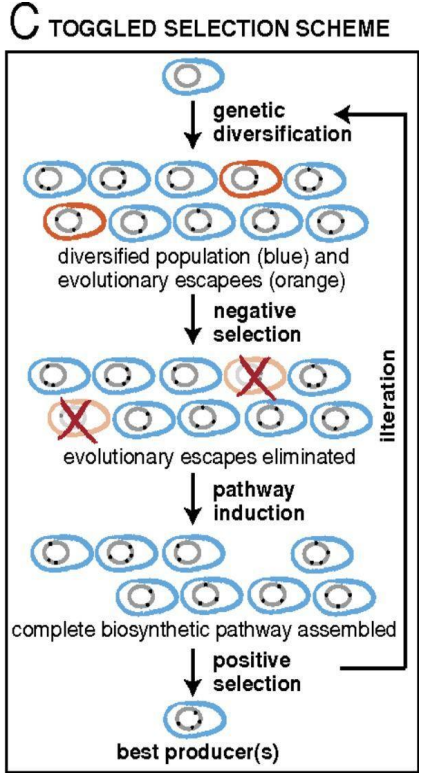
Naringenin

- Pharmacologically useful
- Previous experiments exist against which to measure success

Glucaric acid

- Key chemical in pathways to produce polymers without petroleum
- Unlike naringenin, no previous benchmarks - authors could test system on a new pathway

Combatting “evolutionary escape”



- “**Cheater cells**” survive selection without producing target molecule: mutations that reduce sensor sensitivity
- Leaky selector leads to higher incidence of false positives
- **Operational range:** chemical inducer range that yields production advantage
- **Toggled selection scheme**: alternating negative/positive selection using TolC limits escapees without decreasing operational range
- Resulting cell pool contains best producers out of all cells with optimized pathways

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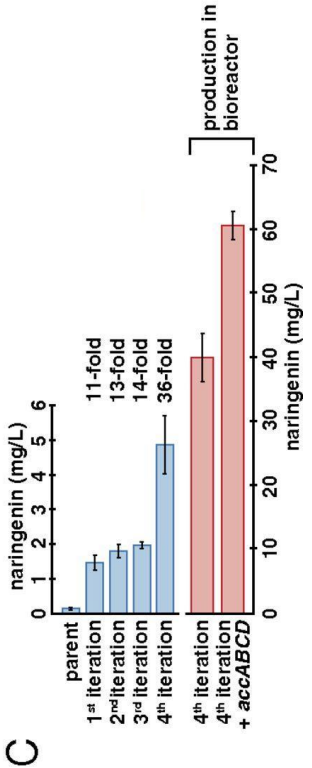
Producing naringenin



- Production limited by malonyl-CoA: sought to optimize malonyl-CoA production through glycolysis, fatty acid biosynthesis, and TCA cycle
- MAGE to control gene expression
- Identified and modified seven genes important in regulation; found many nontargeted mutations during later sequencing
- 4th round titer **36 times higher** than in parent strain; **>400 times higher** in bioreactor with additional mutation

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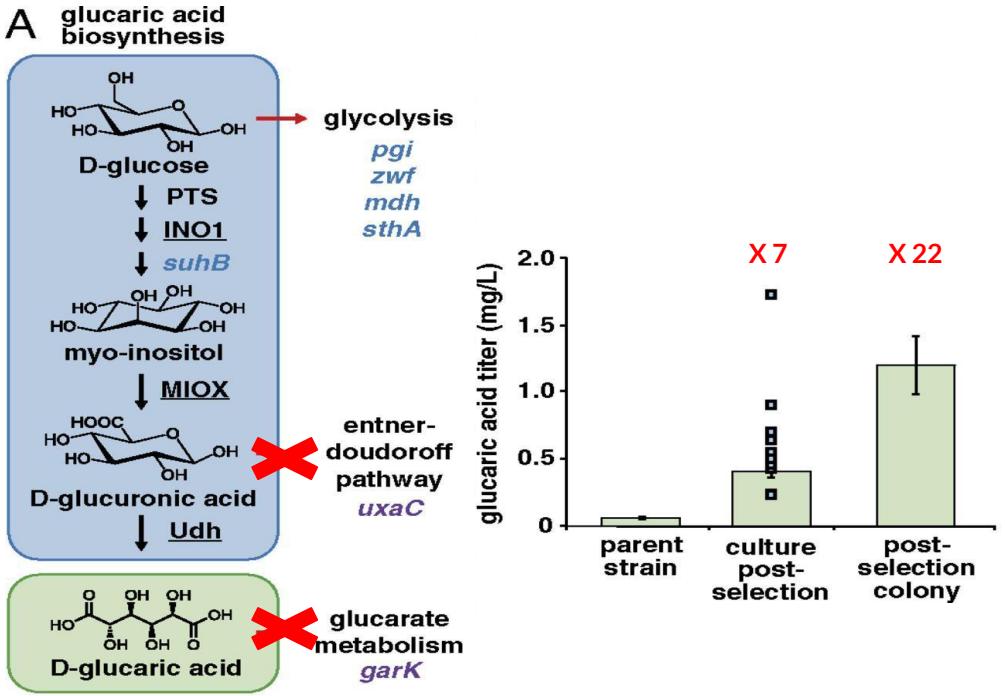
Producing glucaric acid

- Focused on branch point between glucaric acid synthesis and glycolysis
- Knocked out enzymes responsible for glucaric acid and glucuronic acid catabolism
- Found that strain used for enrichment was not optimized
- *E. coli* B strain titer was 300 times higher than parent strain versus 22 times in K strain



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Future work

- Use of this type of sensor selectors to interrogate pathways of potentially useful chemicals
- Design of novel transcription factors to detect metabolic targets
- Utilize branch points to optimize different end points of metabolic pathways
- Investigate gene locations of untargeted mutations for further potential optimization
- Patent pending! George Church’s lab have numerous patents related to microbe genetic engineering

Critiques for Raman et al.

Concerns we would have as a reviewer

- System may not be modular or widely applicable
- Paper could have been seen as two separate works
- Supplementary info is extremely long, could use some refinement

What we appreciated about this paper

- This paper had a large amount of work involved but it was all clearly documented
- Clear explanations of the experiments that lead them to make certain decisions
- Any possible question about mutations or sequences could be found in the supplementary material

Evolution-guided optimization of biosynthetic pathways

Significance

- Microbes can be engineered to produce industrially significant chemicals
- Microbe metabolism engineering can now balance cell viability and productivity

Goals

- Use a toggle switch and FBA to select for best producers of glucaric acid and naringenin

Experiments

- Target pathway to increase key starting material and limit end product catabolism

Results

- Strains with 36 fold increase in naringenin* production and 22 fold increase of glucaric acid**
  * 400 fold increase with additional upregulation
  **300 fold with better suited strain