Washington University in St. Louis
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IN VIVO POPQC – SIMPLE QUALITY CONTROL SYSTEM TO ENHANCE INDUSTRIAL-SCALE MICROBIAL PRODUCTION OF CHEMICALS, BIOFUELS AND PHARMACEUTICALS

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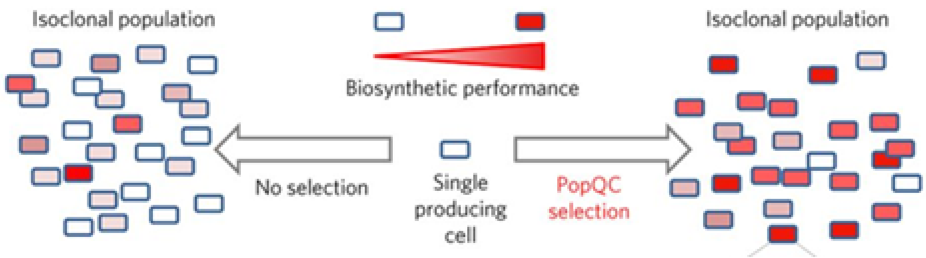
Researchers in Prof. Fuzhong Zhang's laboratory have developed a simple, scalable, broadly-applicable system to enhance fermentation efficiency for biosynthetic production of biofuels, pharmaceuticals, materials and other useful chemicals. Currently, biosynthetic pathways have enabled renewable production of an immense variety of high-value products ranging from simple fuels to intricate natural products. However, overall production efficiency is diminished by nongenetic factors that can cause cell production to vary up to tenfold from cell to cell within an isogenic population. This new genetically-encoded synthetic regulatory tool, called "PopQC", exploits that variation by continuously enriching for highly-productive cells while eliminating low-producing cells.

PopQC utilizes two components, a biosensor and a selection gene that together constantly detect the fermentation product and act as a quality-control kill switch to remove cells with low production. This improves overall biosynthetic performance by optimizing feed-stocks – low-performing microbes are not consuming nutrients that could be used for high-performing cells instead. PopQC could be combined with other genetically based tools to improve biosynthetic yield even further. This system is broadly applicable to enhance biosynthesis for wide range of metabolic pathways, with demonstrated results in *E. coli* for both a chemical/biofuel precursor (free fatty acid) and a pharmaceutical precursor (the amino acid tyrosine).



Overview of PopQC selection: *PopQC confers a growth advantage to high-performing cells, increasing their proportion in the whole population. In the case of PopQC for free fatty acid (FFA) biosynthesis, an FFA-responsive transcription factor acts as the biosensor to regulate the expression of a tetracycline resistance selection gene. In the presence of tetracycline, high-performance (dark red) cells can outcompete low-performance (light red and white) cells to dominate the population, thereby increasing overall productivity 3-fold.*

Stage of Research

The inventors validated this system using two alternative design principles in *E. coli* cells engineered to produce two different compounds (free fatty acids (FFA) and tyrosine). PopQC **increased overall product titers of by 3-fold**. In addition, fed-batch FFA production with PopQC achieved **21.5 g l^{-1} titer and 0.5g $l^{-1}h^{-1}$ productivity** (the highest titer and production rate ever reported for this compound).

Applications

- **Industrial-scale microbial production/fermentation**:
  - end products such as chemicals, pharmaceuticals, nutraceuticals and biofuels
  - demonstrated in FFA (precursor for biofuels and high-volume chemicals) and tyrosine (precursor to pharmaceuticals)
  - PopQC could be incorporated as a stand-alone system or combined with conventional biosynthetic enhancement systems

Key Advantages

- **Enhanced, cost effective biosynthesis**
  - initial experiments demonstrated >300% increase in titers of microbially-produced compounds
  - continuously selects for high-producing cells throughout fermentation
  - likely to have greater impact in industrial-scale production where microenvironments exaggerate nongenetic variations
- **Scalable -** demonstrated in fed-batch FFA production, yielding the highest titer and production rate ever reported for this compound (21.5 g l^{-1} titer and 0.5 g $l^{-1}h^{-1}$ productivity)
- **Simple, broadly applicable design**
  - simple biosensor/selector system can be used to enhance production of a range of compounds, as long as a biosensor is available
  - demonstrated for free fatty acid and tyrosine
  - should be easily combined with traditional production enhancement techniques to enrich performance due to nongenetic variation and further enhance biosynthesis toward theoretical maxima

Publications

- Xiao, Y., Bowen, C. H., Liu, D., & Zhang, F. (2016). Exploiting nongenetic cell-to-cell variation for enhanced biosynthesis. *Nature chemical biology*, 12(5), 339.
- Survival of the hardest-working, *theSOURCE* March 21, 2016

Patents

- Quality control for improved product biosynthesis (PCT Publication WO2017040958)

Website

- Zhang Lab