Cell Mates: Engineering Metabolic Cooperation and Cellular Codependence

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Producing complex compounds burdens organisms

• Done by placing a large system of enzymes and genes in a cell
• Fitness costs – metabolic burden, negative feedback

How can we decrease the fitness costs?

[Diagram of enzymes and genes in a cell]

Artemisinin

[Chemical structure of Artemisinin]

http://www.bifcpresidency.tn.gov.in/arthemis.html
Organisms cooperate in nature to accomplish a task

- Organisms tend to exploit others to obtain nutrients
- Some collaborate to obtain a mutual evolutionary advantage
- Our own microbiome greatly influences health

http://i.telegraph.co.uk/multimedia/archive/01697/fts-bacteria2_1697950c.jpg
Can we engineer organisms to make a compound by harnessing symbiosis?

- Split enzymatic pathway in a way that:
  - Relieves metabolic burden
  - Eliminates negative feedback
  - Enables modular pathways
Synthetic Approaches to Tunable Symbiosis

1. Split Pathway

\[ A \rightarrow B \rightarrow C \rightarrow D \rightarrow E \rightarrow F \]

2. Tuning Population Ratio
   a. Auxotroph System
   b. Toxin/Antitoxin System
Synthetic Approaches to Tunable Symbiosis

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A Model Pathway: Violacein

- produced in nature by *Chromobacterium violaceum*
- known to have antibiotic and anti-cancer properties
- frequently used commercially as a dye
- Easy readout
- produced in a pathway involving 5 different enzymes: VioA-E
Violacein Pathway

ViolaC

protodeoxyviolacein acid

VioD

Non-enzymatic

CO₂, H₂O

O₂

Non-enzymatic

Violacein

protodeoxyviolacein

VioE

VioB

VioA
Violacein Pathway
Split Violacein Pathway

VioA → VioB → VioE → protodeoxyviolaceinic acid → VioD → VioC → violatesin

Non-enzymatic steps:
- \( \text{O}_2 \rightarrow \text{CO}_2, \text{H}_2\text{O} \)
- \( \text{CO}_2, \text{H}_2\text{O} \rightarrow \text{O}_2 \)
Splitting the Violacein Pathway

Strain 1

Vio A -> Vio B -> Vio E

Intermediate

Strain 2

Vio D -> Vio C

Violacein
Violacein Production and Extraction
Violacein Production and Extraction

ETHANOL EXTRACT

Monocultures (Strain 1) (Strain 2) Co-culture (Strain 1 & 2)
Growth of Co-Culture vs Operon

Operon growth

Coculture growth

OD$_{600}$ vs time for uninduced and induced conditions.
Violacein is produced by *E. coli* co-culture

![Graph showing the amount of violacein produced by different strains.](image-url)
How can we regulate the two strains?

- Optimal production using multiple strains require control over population ratio
- 2 codependent systems
  - Essential compound exchanged between strains
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Tuning Auxotroph System Through Inducible Promoters

Cells and system given to us by the Lin Lab of the University of Michigan
Tuning Auxotroph System Through Inducible Promoters

Tyrosine auxotroph (tyr⁻)

Tryptophan auxotroph (trp⁻)

Arabinose

Sodium Propionate

plasmid allows trp to be exported out of the cell

plasmid allows tyr to be exported out of the cell

Cells and system given to us by the Lin Lab of the University of Michigan
Simulating Auxotroph System Dynamics

• Predict which ratios are possible or impossible
• Accurately predict and control the system to maximize production
Simulating Auxotroph Populations

\[ \frac{dn_1}{dt} = \mu_1 \cdot n_1 \]

- \( n \) = number of cells
- \( c \) = concentrations of cross-fed metabolites
- \( \alpha \) = auxotroph export rate
- \( \beta \) = cellular requirement for essential metabolites (how much metabolite cells need to survive)
- \( K \) = a threshold that determines the half-way value of the maximum growth rate
- \( k \) = max growth rate of *Escherichia coli*
- \( \mu \) = rate at which cells divide

Kerner et al.
Simulating Amino Acid Concentrations

\[ \frac{dc_1}{dt} = \alpha_2 n_2 - \beta_1 \cdot \frac{dn_1}{dt} \]

- \( n \) = number of cells
- \( c \) = concentrations of cross-fed metabolites
- \( \alpha \) = auxotroph export rate
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- \( K \) = a threshold that determines the half-way value of the maximum growth rate
- \( k \) = max growth rate of \textit{Escherichia coli}
- \( \mu \) = rate at which cells divide

*\( \alpha \) is a controllable parameter

Kerner et al.
Modeling: Amino Acid Export Rates
Tune Cell Ratio

Export rate of tryptophan ($\mu$mol/gDM-hr)  Export rate of tyrosine ($\mu$mol/gDM-hr)
Modeling: Amino Acid Export Rates

Tune Cell Ratio

Export rate of tyrosine:
10 \( \mu \text{mol/gDM-hr} \)
Auxotroph Co-Culturing Shows Tuning Works in a Small Range of Inducer Conditions
Synthetic Approaches to Tunable Symbiosis

1. Split Pathway
   - A → B → C → D → E → F
   - A → B → C
   - D → E → F

2. Tuning Population Ratio
   a. Auxotroph System
   b. Toxin/Antitoxin System
Toxin/Antitoxin System

• Three types:
  o Type 1 - small RNA antitoxin binds to toxin-coding mRNA
  o Type 2 - protein antitoxin binds to protein toxin
  o Type 3 - protein toxin bound by RNA molecule

• Two or more closely related genes that code for a "poison" and its "antidote."
How could toxin/antitoxin pairs regulate strain population?
After two hours of incubation, we induced with IPTG and grown at 30°C overnight.
The next day...

Pellet cells

150mL LB + control cells

150mL LB + antitoxin cells

150mL LB + toxin cells

Filter

LB + control supernatant

LB + antitoxin supernatant

LB + toxin supernatant
Antitoxin cells

Diluted control supernatant

Diluted antitoxin supernatant

Diluted toxin supernatant
Growing antitoxin cells in presence of toxin has a negative effect on growth

![Graph showing average growth of antitoxin cells](image)

- Blue line: in control sup.
- Pink line: in antitoxin sup.
- Green line: in toxin sup.

**Y-axis:** average OD

**X-axis:** time (hrs)

Graph illustrates the growth trend over time under different conditions.
Parts Registry Contributions

- Construct coding for YefM (anti-toxin) behind an IPTG inducible T7 promoter
  - Part: BBa_K726012
- Construct coding for YoeB (toxin) behind an IPTG inducible T7 promoter
  - Part: BBa_K726014
- Construct coding for three enzymes (VioA, VioB, VioE) behind an IPTG inducible T7 promoter
  - Part: BBa_K726016
- Construct coding for two enzymes (VioD, VioC) behind an IPTG inducible T7 promoter
  - Part: BBa_K726016
Summary of Results

- We have successfully **split a model pathway** between two strains of cells
  - *E. coli* co-cultures are capable of working together to produce violacein
  - The co-culture is as efficient as a monoculture and could be tuned to maximize production
- We have analyzed an auxotroph system and shown through our modeling that tuning cell ratios is feasible
- We have shown that toxin/antitoxin system is potentially a **novel intercellular communication system** and could be used to tune population ratios
Future Directions

• Continue working with the toxin/antitoxin system to create a simple, reliable method for tuning cell ratios
Future Directions

- Incorporate split pathway into our tuning systems to identify appropriate ratios to maximize production and efficiency
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Simulating Toxin/Antitoxin System Populations

- $n =$ number of cells
- $T =$ amount of toxin in cell
- $A =$ amount of antitoxin in opposite cell
- $C =$ amount of compound formed by toxin-antitoxin binding
- $b =$ synthesis rate of toxin/antitoxin ($b_T/b_A$)
- $d =$ degradation rate of toxin/antitoxin ($dT/dA$); also complex ($dC$)
- $K =$ a threshold that determines the half-way value of the maximum growth rate
- $k_m =$ max growth rate of $E. coli$
- $k_n =$ minimum growth rate of $E. coli$ (negative)
- $\mu =$ ($T; k, K$) rate at which cells divide

\[
\frac{dn_1}{dt} = \mu_1 \cdot n_1
\]

\[
\mu_1 = (k_{m1} - k_{n1}) \cdot \left(\frac{K_1}{K_1 + T_1}\right) + k_{n1}
\]
Simulating Changes in Toxin, Antitoxin, and Complex

\[
\frac{dT_1}{dt} = b_{T1} - d_{T1}T_1 - A_1T_1r_1
\]

- Synthesis rate of toxin
- Amount of toxin degraded
- Rate at which complex is made

\[
\frac{dA_1}{dt} = b_{A1}n_2 - d_{A1}A_1 - A_1T_1r_1
\]

- Amount of antitoxin synthesized
- Amount of antitoxin degraded

\[
\frac{dC_1}{dt} = A_1T_1r_1 - d_{C1}C_1
\]

- Amount of complex degraded

**Variables:**
- \( n \) = number of cells
- \( T \) = amount of toxin in cell
- \( A \) = amount of antitoxin in opposite cell
- \( C \) = amount of compound formed by toxin-antitoxin binding
- \( b \) = synthesis rate of toxin/antitoxin (\( bT/bA \))
- \( d \) = degradation rate of toxin/antitoxin (\( dT/dA \)); also complex (\( dC \))
- \( K \) = a threshold that determines the half-way value of the maximum growth rate
- \( km \) = max growth rate of \( E. coli \)
- \( kn \) = minimum growth rate of \( E. coli \) (negative)
- \( \mu \) = \((T;k,K)\) rate at which cells divide
Wavelength Scan of Violacein Cultures
Supernatant addition might show antitoxins being exchanged between cells.

**Toxin producing cells**
little/no growth when induced

**Toxin cells** uptake antitoxins and continue to grow
average growth of control cells

- in control sup.
- in antitoxin sup.
- in toxin sup.