The Co-Cultivators
Bacterial Consortia for the Improvement of Consolidated Bioprocessing Techniques
Consolidated Bioprocessing

- Cellulose as substrate
  - Monocultures
  - Co-cultures
  - Bacteria choice for CBP

Designing our system

- Modeling
- Naïve co-culture
- Measurement
- Circuit design

Implementation Details

- *C. Hutchinsonii* as a new chassis
- Biodiesel
Cellulose is lying around in abundance

- 120 million tons of corn stover
- Cheap
- Not a food source

Enzymes → Energy → $$$

... but difficult to process

Corn Stover, Agriculture and Agri-food Canada, 2008.
CBP uses a single reactor for all production steps.

The CBP State of the Art relies on monoculture-based processing, which isn’t reliable.
Crystalline Cellulose
Co-cultures are better than monocultures

Co-cultures allow for specialty, robustness, and are inspired by nature.
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Co-cultures allow for specialty, robustness, and are inspired by nature.
Cytophaga hutchinsonii: degrades cellulose, aerobic, sequenced genome and transformable
E. coli:
well characterized and produce a variety of products
Our product: biofuel
Preliminary Modeling Suggests that Co-Culture can be Improved with Communication

\[
\frac{dX_1}{dt} = \frac{3}{2} X_1 \left( 1 - \frac{X_1}{5} \right) + X_2
\]

\[
\frac{dX_2}{dt} = 5X_2 \left( 1 - \frac{X_2}{25} \right) - 3X_1
\]
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Modeling to Predict Co-Culture Dynamics
**Modeling to Tune Populations - Forward Design**

- **Substrate**
- **Population 1**
- **Population 2**
- **Product**

**Graph:**
- Y-axis: Concentration
- X-axis: Time
- Lines:
  - Green: Substrate (decreasing)
  - Red: Population 1 (rising)
  - Blue: Population 2 (rising)
  - Orange: Product (rising)
Start with the Flux Balance Analysis

### Stoichiometric Matrix

- $\mathbf{A}$
- Steady state
- $\mathbf{A} \mathbf{v} = 0$
- $\mu = \mathbf{w}^T \mathbf{v}$

Source: BiGG database, Orth et al. 2011
Then consider extracellular dynamics

Flux Balance Model
Max $\mu = w^Tv$

s.t.: $Av = 0$

$lb < v < ub$

$\mu, v_p$

Uptake/Secretion Kinetics

$v_s = f_s(S, P)$

Extracellular Mass Balances

$\frac{dX}{dt} = \mu X$

$\frac{dS,P}{dt} = \sum v_{si} X$
We Overcame Many Significant Challenges in Implementing Our Models

Valid metabolic networks

Kinetic parameters

\[ v_0 = \frac{V_{\text{max}}[S]}{K_m + [S]} \]

Computationally feasible

Non-native pathways

openCOBRA

openCOBRA

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- Modeling
- Naïve co-culture
  - Grow *E. coli* and *C. hutchinsonii* together
  - Separate populations
  - Analyze carbohydrates
- Circuit design

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Naïve Co-Culture to Tell Us What to Improve

Filter paper

C. hutchinsonii

E. coli

Salts

drpinna.com

humboldtmfg.com

Standardsingenomics.org

lbc.msu.edu
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Designing our system

• Modeling
• Naïve co-culture
  • Growing *E. coli* and *C. hutchinsonii* together
  • Measurement
• Circuit design

Implementation Details

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We developed a method of distinguishing cells by shape.

C. Hutchinsonii

E. Coli
We developed a method of distinguishing cells by shape.

http://ucflow.blogspot.com/
We developed a method of distinguishing cells by shape.
Carbohydrate Analysis: Sulfuric Acid-Phenol Method

Naïve Co-culture Data

Concentration [g/L] vs. Time [h]

Total Carbohydrates Data
Total Carbohydrates Fitted Data

Standards
Naïve Co-culture Data

Absorbance 490 nm vs. Concentration (%wt/v)

Round 1
Round 2
Round 3
Average
Linear Regression
Modeling Predicts Naïve Co-Culture Data

Data

- Total Carbohydrates
- C. hutchinsonii
- E. coli

Model

- Biomass C. hutchinsonii
- Biomass E. coli
- Filter paper
- Glucose
- Xylose
- Cellodextrins (2-7)
- Xylo-oligosaccharides (2-7)
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Implementation Details
- *C. Hutchinsonii* as a new chassis
- Biodiesel
Pros of Continuous Culture
• Higher productivity
• No turnover time

Cons of Continuous Culture
• Susceptible to genetic instability
• Susceptible to contamination
• Impractical in industry

Changing Focus from Continuous to Batch Culture
We used a toxin and antitoxin system to modulate C. hutchinsonii population size.
We used the Lux quorum sensing system for inter-bacteria communication.

AHL

Expression!
Circuit design tethers *C. Hutchinsonii* growth to *E. Coli*

*C. hutchinsonii*

- LuxR
- GFP
- RelE
- RelB

*E. Coli*

- AHL
- LuxI
- RFP
- pLac (biodiesel (5 genes))

Simple sugars
Modeling predicts circuit failure and success modes.

Success: *C. Hutchinsonii* to *E. Coli* ratio increased.
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Engineering *C. hutchinsonii* Requires Plasmids

Basic Plasmid

- Selectable Marker
- Cloning Site
- ori
Origin of Replication Part Design

- PCR amplified chromosomal origin of replication (oriC)
- BioBrick and MoClo compatible
- Part# Bba_K1705010

C. hutchinsonii Genome
Inserting oriC in both Cm$^R$ and Kan$^R$ backbones:
- Cm resistance to 10 ug/mL
- Kan resistance at 30 ug/mL
C. hutchinsonii Accomplishments

• Opened new, interesting chassis
  • Culture and transformation methods
  • Modularized origin of replication (Bba_K1705010)
  • Selection with Kan and Chlor

• Future directions
  • Native constitutive promoter (Bba_K1705012)
  • Native RBS (Bba_K1705011)
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Producing Biodiesel with E. coli

Sugars → ‘tesA (BBa_K1705002) → FadD (BBa_K1705003) → Acyl-coA → atfA (BBa_K1705001) → Fatty Acid Ethyl Esters (FAEEs)

pdc adhB (BBa_K1705000) → Ethanol
Developed a method to detect biodiesel.

90% Methanol Standards

- Ethyl Palmitate
- Palmitic Acid

Developed a method to detect biodiesel.
We developed a method to separate a fatty acid from its ethyl ester.
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• C. Hutchinsonii as a new chassis
• Biodiesel
• Characterized co-culture
• Characterized co-culture
• Characterized co-culture

• Designed population control system
• Characterized co-culture

• Designed population control system

• Progress toward implementation
• Characterized co-culture

• Designed population control system

• Progress toward implementation
• Characterized co-culture
• Designed population control system
• Progress toward implementation
• Collaboration
• Addresses issue of population instability

• Modular system

• New use of cellulosic waste

• Future challenges:
  • Scalability
  • Genetic instability
Acknowledgements
Separating Populations

![Graph showing data points and distributions](image)

- Forward Scatter Height

![Histogram showing data distribution](image)
E. coli Growth on Dead C. hutchinsonii
Naive Co-Culture Data

Naive Co-Culture Growth Curve

E Coli
C Hutch

cells/mL

days

$10^4$ $10^5$ $10^6$ $10^7$

0 1 2 3 4 5 6 7 8 9 10
NAIVE CO-CULTURE DATA

- Contamination (media, no cells)
- 4.1 conditioned hutch + coli
- 5.1 unconditioned hutch + coli
- 5.2 unconditioned hutch + coli
- 2.2 "just" coli
- 3.1 "just" hutch
- 4.2 conditioned hutch + coli
- 2.1 "just" coli
- 3.2 "just" hutch