Ultrafast Growing Cyanobacteria for Fuel and Chemical Production

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Track 5: Synthetic Biology & Genomic Research
“Microbial and Synthetic Approaches to H2 Metabolism for CO2 Utilization”
2017 World Congress on Industrial Biotechnology
Montreal, Canada
Moving Beyond Traditional Chassis

Focus on C&E fluxes, regulatory networks architecture, adaptive responses – enable rational design and prediction
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- Traditional chassis (e.g., E. coli) cannot be readily engineered for photosynthesis, adaptations to extremes

Courtesy of Dr. Victoria Work
Moving Beyond Traditional Chassis

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More logical - improve the quality of photoautotrophic model organisms!
Why Cyanobacteria?

- The most ancient photosynthetic organisms
- Diverse & ubiquitous
The most ancient photosynthetic organisms

Diverse & ubiquitous

Variety of useful traits

- Easily cultivated, genetically tractable
- Wealth of genomic data and tools

Why Cyanobacteria?
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- The most ancient photosynthetic organisms
- Diverse & ubiquitous
- Variety of useful traits
  - Easily cultivated, genetically tractable
  - Wealth of genomic data and tools
- *Synechococcus* sp. PCC 7002, unicellular euryhaline strain
  - Model for studying photosynthesis (light harvesting and PET)
  - Tolerant to high light intensities, $O_2$ concentrations and oxidative stress
  - One of the fastest growth rates (0.2-0.32 h$^{-1}$)

(A) Light and (B) electron micrograph of wild-type *Synechococcus* PCC 7002 (Bryant’s Lab)
Enhancement of biological productivity requires the ability to manipulate reductant fluxes and carbon allocations.
Synechococcus 7002: Model for Studying Photoautotrophic Productivity

- Enhancement of biological productivity requires the ability to manipulate reductant fluxes and carbon allocations

- Formulation of stoichiometric relationships (reductant production vs consumption) leads to a structured metabolic model
**Synechococcus 7002: Model for Studying Photoautotrophic Productivity**

- Productivity enhancement requires the ability to manipulate reductant fluxes and carbon allocations.

- Formulation of stoichiometric relationships (reductant production vs consumption) leads to a structured metabolic model.
Models can simulate growth as function of light quantity and quality and predict electron transfer pathways for optimal productivities.

From Vu et al., 2012. Vu et al., 2014.
Modeling Reductant Partitioning and Carbon Fluxes

► Models can simulate growth as function of light quantity and quality and predict electron transfer pathways for optimal productivities

► In silico mutagenesis tests gene functionality and provides hypotheses for pathway optimization

From Vu et al., 2012. Vu et al., 2014.
Controlled Cultivation: LED PBR

- Custom-made LED enclosure for narrow-spectrum light compatible with commercial systems (BioFlo)
- 630 & 450/680 nm – phycobilisomes & chlorophyll
- Computer feedback control of incident/transmitted light to control light climate
- Batch and continuous cultivation modes (chemostat, turbidostat, auxostat)

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**Turbidostat:**
Cell growth is unrestricted, i.e., $\mu \rightarrow \mu_{\text{max}}$ (condition specific)

Effect of irradiance (I) on specific growth rate (μ)

From Bernestein et al., 2016. mBio
Photophysiology of Fast Growth

- Effect of irradiance (I) on specific growth rate (µ)

- Response to O₂ stress

From Bernestein et al., 2016. mBio
Chlorophyll fluorescence induction kinetics (example of PAM fluorometry)
Electron Transfer at High Irradiances

Chlorophyll fluorescence induction kinetics (example of PAM fluorometry)

High fluxes of cyclic electron transport correspond to fast growth at high light levels

(A) rETRmax

Syn 7002
Cyan 51142
Electron Transfer at High Irradiances

Chlorophyll fluorescence induction kinetics (example of PAM fluorometry)

- High fluxes of cyclic electron transport correspond to fast growth at high light levels.
- These fluxes dissipate quickly via cyclic ET during fast growth and may serve to protect PS II from damage.
Genetic Acclimation to Oxidative Stress

From Bernestein et al., 2016. mBio
Genetic Acclimation to Oxidative Stress

- Increase in growth-related functions and biosynthetic pathways (black)

From Bernestein et al., 2016. mBio
Acclimation to Oxidative Stress (Cytological)

- **0.030/h growth rate**
  - Avg. cell volume = 3.43 µm³
  - n=104

- **0.086/h growth rate**
  - Avg. cell volume = 4.84 µm³
  - n=107

- **0.169/h growth rate**
  - Avg. cell volume = 9.96 µm³
  - n=65

Average cell volume of *Synechococcus* 7002 increases up to 200% with increased specific growth rates.
Genetic Acclimation to Oxidative Stress

- Increase in growth-related functions and biosynthetic pathways (black)
- Decrease in light acquisition pathways and antennae (blue)

From Bernestein et al., 2016. mBio
Genetic Acclimation to Oxidative Stress

- Increase in growth-related functions and biosynthetic pathways (black)
- Decrease in light acquisition pathways and antennae (blue)
- Increase of cyclic electron transfer and $C_i$ transport under high light (green)

From Bernenstein et al., 2016. mBio
Genetic Acclimation to Oxidative Stress

- Increase in growth-related functions and biosynthetic pathways (black)
- Decrease in light acquisition pathways and antennae (blue)
- Increase of cyclic electron transfer and C\textsubscript{i} transport under high light (green)
- No changes in central C metabolism or stress response

From Bernestein et al., 2016. mBio
Knock-outs of genes encoding 2-OGDC, SSADH and SDH have been made, so TCA cycle does not have to be complete — but it is. Genes also found in some archaea and clostridia.

**The Cyanobacterial TCA Cycle: complete at last...**

**Fumarase**

**Succinic semialdehyde dehydrogenase**

*SynPCC7002_A2771*

**NADP**

**2-oxoglutarate decarboxylase**

*SynPCC7002_A2770*

From SSA to 1,4-butanediol

1: 2-oxoglutarate decarboxylase
2: succinyl-coA synthase
3: CoA-dependent succinate semialdehyde dehydrogenase
From SSA to 1,4-butanol

1: 2-oxoglutarate decarboxylase
2: succinyl-coA synthase
3: CoA-dependent succinate semialdehyde dehydrogenase
4: 4-hydroxybutyrate dehydrogenase
5: 4-hydroxybutyryl-CoA transferase
6: 4-hydroxybutyryl-CoA reductase
7: aldehyde dehydrogenase (native)

1,4-Butanediol production in *Synechococcus* sp. PCC 7002

CONCLUSIONS

- *Synechococcus* 7002 is a robust platform which maintains fast growth rates high irradiances and extreme O$_2$ concentrations.

- Proof-of-principle studies demonstrate the ability to manipulate fluxes of carbon and energy through environmental or genetic perturbations.

- Availability of an experimentally-validated genome-scale model and systems-level data increase the predictability of metabolic & gene network engineering.

- Next steps need to demonstrate productivity improvements on the “per quantum” basis which will lead to the design of new strains using metabolic modeling and synthetic biology approaches.
ACKNOWLEDGEMENTS

**Pacific Northwest National Lab:**
- Dr. Ryan McClure
- Dr. Allan Konopka
- Dr. Matthew Melnicki
- Dr. Grigoriy Pinchuk
- Dr. Hans Bernstein
- Eric Hill
- Dr. Tom Metz
- Leo Kucek

**Penn State University:**
- Dr. Donald Bryant

**Colorado School of Mines:**
- Dr. Matt Posewitz
- Victoria Work

**University of Wisconsin:**
- Dr. Jennifer Reed
- Trang Vu
- Josh Hamilton

**Burnham Inst. Medical Research:**
- Dr. Andrei Osterman
- Dr. Jessica DelIngeniis

**Funding by:**
- U.S. DOE BER Genomic Science Program