

Algal Biology Breakout Session

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Algal Biology

- The Algal Biology break out session, due to its large subject size, was separated into 3 major sub-sessions:
 - Subtopic #1: Strain isolation, selection and screening
 - Subtopic #2: Cell biology
 - Subtopic #3: Genomics and systems biology
- The overall goals of algal biology are to:
 - Isolate, characterize and genetically improve oleaginous algae that can be used in commercial scale biofuel production
 - Develop a fundamental understanding of algal cell biology including photosynthesis, carbon partitioning, lipid synthesis and deposition, and cell wall composition & ultrastructure
 - Use a genomics/systems biology approach to study algal metabolic pathways of lipid synthesis



Subtopic #1: Strain Isolation, Selection and/or Screening

- Isolation and characterization of naturally-occurring species /strains
 - Natural habitats: marine, freshwater, brackish/saline, wastewater and extreme environments
 - Identification of criteria for screening: examples, maximum specific growth rate; maximum cell density; tolerance to CO₂, temperature, light grazers, and pH salinity, susceptibility to predators, co-product formation)
 - Approaches to screening: Agar plating, FACS, microtiter plates, test tubes, flasks, carboys, glass columns, shaking vs mixing, continuous vs light-dark cycle, use of different media.
 - Parameters and methods for growth measurement: dry weight, optical density, chlorophyll, cell counts volumetric biomass productivity (g/L/day), areal biomass productivity (g/m²/day)
 - Parameters and methods for analysis of lipid and other desirable products: general compositional analysis (protein, lipid and CHO), rapid oil analysis (NIR, NMR), neutral lipid, polar lipids, fatty acid profile, gravimetric vs neutral lipid specific dyes, spectroscopy vs flow cytometry, LC/MS, GC, GC-GC/MS



Subtopic #1: Strain Isolation, Selection and/or Screening

- Development of novel concepts and approaches for strain screening, selection and characterization.
- Role of algal culture collections
 - Strain deposition
 - Cryopreservation/viability issues
 - Serial passaging (concerns about genetic drift)
 - Maintenance protocol standardization
 - Strain identification and classification



Subtopic #1: Strain Isolation, Selection and/or Screening

Discussion centered around:

- Naturally occurring strains or hybrid/GMOs? In other words, is strain development a topic to consider for commercialization?
- Barriers include basic guidance in “what are we screening for” : is it TAG? All lipids? Growth characteristics? Environmental tolerances (CO₂, temperature, pH etc)?
- Serial samples and the selective pressure on cultures: there are issues, but it is unknown if this affects lipid synthesis. An unknown is the relation of laboratory selective pressures to outdoor culture selective pressures.
- A definition and characterization and comparison of baseline physical environments (including seasonal variability and community composition) is necessary. Lessons from the ASP should be noted: promising species DID NOT grow in ponds.
- The role of metagenomics to identify community composition has promise and challenges.
- In the end, the key screening characteristic may be consistency.



Subtopic #1: Key Challenges

Top challenges for isolation, selection and/or screening:

1. What algae specifications are we looking for that meets fuel needs? Is bioprospecting or bioengineering the right path? What are the key characteristics to look for?
2. Isolation and screening of organisms and communities in a fast, high-throughput, and low labor manner. Related to screening: *in situ* organisms may be different than laboratory cultures. Solid suspension analysis?
3. Database infrastructure: consistent, consolidated, and robust publicly available information is not available
4. The ownership of strains and cultures, especially those collected from national parks, requires consideration
5. Strain stability/community stability and resilience: How to quantify and measure? Disease resistance? Small scale wells?
6. Define physical environment that organisms are screened from and develop a community stability methodology.
7. Consider/screen for excreted materials.
8. Consider macroalgae.



Subtopic #1: Recommendations

Priorities:

- Develop screening standards and a database infrastructure that contains relevant information about different algal strains
- Develop high-throughput methods for isolation and screening organisms from a variety of unique environments (aquatic, airborne and terrestrial)
- Baseline regional environmental cases



Subtopic #2: Cell Biology

1. *Photosynthesis*

- Photosynthetic efficiency
 - Theory, assumptions, and calculation
 - Projection of theoretical maximum biomass productivity at various geographical locations and climate conditions
 - Modeling of biomass/product yield potential
 - Effects of environmental factors on photosynthetic efficiency and biomass/oil yield potential
- Photosynthetic regulation of lipid synthesis
 - Electron transport chain/PQ/antenna pigments
 - ATP/NADPH

2. *Metabolic carbon fluxes and partitioning*

- Biosynthesis and regulation of sugar/starch synthesis
- Photosynthetic carbon Partitioning into storage lipid
- Signal transduction and regulation of carbon partitioning
- Modeling of carbon fluxes and partitioning



Topic #2: Cell Biology (continued)

3. *Lipid synthesis and regulation*

- *De novo* fatty-acid and lipid synthesis and regulation
- Alternative pathways of storage lipid synthesis
- Conversion of membrane lipids/other components into storage lipids
- Enzymology of relevant biosynthetic pathways (TAGs, isoprenoids others)
- Pathways/mechanisms of lipid secretion
- Biogenesis, composition, structure and function of lipid bodies
- Interactions among the organelles (e.g., chloroplast, Golgi bodies, ER, mitochondria and lipid bodies) related to storage lipid accumulation
- Ultrastructure of lipid bodies and lipid body formation
- Relationships between oxidative stress and cell division and storage lipid formation
- Molecular and cellular mechanisms and physiological role of storage lipid synthesis



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Topic #2: Cell Biology (continued)

4. Algal cell walls and cytoplasmic membrane

- *Composition and ultrastructure*
- *Changes during growth and effect of stress conditions*
- *Implication for algal harvest, oil extraction, and conversion of cell walls to other forms of biofuels and biomaterials*

5. Biohydrogen

- *Direct biophotolysis*
- *Indirect (anaerobic fermentative) biophotolysis,*
- *Oxygen sensitivity of nitrogenase and hydrogenase activities.*



Subtopic #2: Cell Biology

Recommended Research Milestones

- **Photosynthesis**
 - *Determine theoretical and practical maximum photosynthetic efficiencies and lipid production potential*
 - *Identify key regulators of photosynthesis affecting lipid formation*
 - *Genetic manipulation of photosynthetic mechanism for enhanced lipid production (e.g., reduce antenna size, enhance carbon concentrating mechanism, change ATP/NADPH and PQ pool)*
- **Lipids**
 - *Identify lipid pathways and key regulators*
 - *Investigate membrane lipid degradation, conversion, and lipid body formation*
- **Carbon partitioning**
 - *Analyze photosynthetic carbon fluxes under various growing conditions*
 - *Regulate carbon partitioning into storage lipid*
- **Stress response**
 - *Determine signaling and regulatory networks controlling cell division and storage lipid formation*
- **Cell wall and cytoplasmic membranes**
 - *Characterize composition and structure of cell wall and cytoplasmic membranes*
 - *Genetically modify cell wall properties to optimize cell harvesting, lipid extraction, and conversion of cell walls to fuels and other materials*




Additional comments from full breakout discussion

- The *arabidopsis* model for plants may not directly apply to algae, because algae have multiple evolutionary origins
- At least 3-5 algal model organism need to be studied because of their diverse phylogenetic background
- Functional genomics, including annotation of algal genomes, need to be emphasized. Gene annotation of representative model algal organisms is needed



Subtopic #3: Genomics and Systems Biology Barriers and Goals

- **3.1** The green alga *Chlamydomonas reinhardtii* and the diatom *Thalassiosira pseudonana* should be considered as the eukaryotic model systems for investigating synthesis and regulation of lipid. Both have sequenced genomes and genetic manipulation tools.
- **3.2** *Only ten algal genomes currently completed (20 in the pipeline):* Development of robust criteria for selecting algal organisms for genome sequencing/genome annotating efforts.
- **3.3** *Fundamental understanding of algal metabolic pathways is inadequate. Decision tree for carbon partitioning in algae is largely unknown:* Establishment of an integrated systems biology and bioinformatics framework to develop a fundamental understanding of carbon partitioning in algae.
- **3.4** *Current genetic toolbox for algae is insufficient.* Adapting current genetic tools/synthetic biology systems for metabolic engineering of model algal organisms.



Subtopic 3.1 (Algal Model Systems)

- **Goal:** Development of algal model systems
- **Key challenges:** How many model systems do you choose (1-5)? Choosing too many could dilute the effort. Decision depends on fuel/intermediate to be produced (i.e., H₂, lipids, carbohydrates, ethanol), availability of genetic tools and specific phenotypic traits (high lipid, high growth rate, secretion of oil, high photosynthetic rates, tolerant of high CO₂). Need to consider photoautotrophs and heterotrophs. Need to also consider including eucaryotic algae and cyanobacteria in the candidate pool.
- **Strategies:** Choose several model systems and use these to carry on into the integrated systems biology approach. Should look to include algae that excrete intermediates (lipids, hydrocarbons, sugars, others). Secretion of products could simplify the process (eliminates the need to harvest and extract). Should *Botryococcus* be a model system? Need to closely coordinate with those performing prospecting and identifying useful traits. Should also not ignore those strains that can be used in heterotrophic applications.
- **Key Milestones:** Near term; choose at least one green alga and one diatom. Longer term might be able to move several (5?) model systems forward in parallel



Subtopic 3.2 (Algal Genome Sequencing)

- **Goal:** Development of robust criteria for selecting algal organisms for genome sequencing/genome annotating efforts
- **Key challenges:** Prioritization of algal sequencing projects; Completing algal projects given other JGI sequencing priorities. Annotation is a huge issue in making genome information useful –up to 40% of ORFs have an unknown function. Venture capital will not support long term genome research, need government support. Bioinformatics is not well developed to take advantage of the genomic information – this will need lots of informatics support.
- **Strategies:** Coordinate early with OBER. Bioinformatics training at the university level should be encouraged. Need to study diverse species, for example, secondary (diatoms, red algae) and primary symbionts (greens). Need much more comparative analysis. A comparative analysis between diverse and closely related species will identify common and diverse principles (may be able to identify stress factors/triggers).
- **Key Milestones:** Algal biofuels community needs to speak with one voice; should develop streamlined annotation methods. Develop close interactions between biologists and computationalists.
- **Required Investment:** More than 20 algal species are currently in the pipeline at JGI; significantly more (100?) need to be identified for sequencing.



Subtopic 3.3 (Integrated Systems Biology Framework)

- **Goal:** Establishment of an integrated systems biology and bioinformatics framework to develop a fundamental understanding of carbon partitioning in algae
- **Key challenges:** Fundamental understanding of algal metabolic pathways and decision tree for carbon partitioning in algae is largely unknown.
- **Strategies:** New sequencing technology (454) will make transcriptome data better. Strain selection should look for traits (i.e., lipid abundance, photosynthetic capacity, secretion of lipids, etc) and then funnel these through systems analysis. Identification of important traits will be highly dependent on the type of fuel or chemical intermediate under development.
- **Key Milestones:** Longer term: Need to develop an integrated architecture for algal *systems biology (soup to nuts) i.e.*, transcriptomics, proteomics, metabolomics and lipidomics. Nearer term: Need to take a “reverse engineering” approach (identify trait [ex. specific lipid profile]>identify proteins that control this trait>understand the expression and regulation of these proteins)
- **Investment:** Need a huge commitment from JGI; \$500M-\$1B over 5 years



Subtopic 3.4 (Development of Metabolic Engineering Tools)

- **Goal:** Development of next generation genetic tools & synthetic biology systems for metabolic engineering of model algal organisms.
- **Key challenges:** Developing a universal (for a general class) genetic toolbox that works with a broad spectrum of species such as green algae and diatoms.
- **Strategies:** Toolbox should include efficient transformation, gene replacement, RNAi and insertional mutagenesis methods; including the identification of suitable promoters, selectable markers, and vectors. Viral contamination could be a huge issue, will need to engineer viral resistance. The availability of transformation systems/technology will help dictate strains but shouldn't be a prerequisite for genomics.
- **Key Milestones:** In order to develop effective genetic manipulation tools, we will need a critical mass to accomplish this in any given species.



Interfaces

- Cultivation
- Harvesting/Dewatering
- Extraction/Fractionation
- Policy/Regulatory (GMOs)
- Co-products



Contentious/Controversial Topics

- 2-tiered approach to screening: organisms that cultivate well and organisms that have desirable properties or traits
- Consistent production of a feedstock.
- Separation of lipid accumulation and growth: has it been achieved through pH management/TCA cycle deactivation or not?
- What is the role of communities? How can one isolate, screen, and characterize communities?
- Development of phototrophic vs heterotrophic algal oil production.
- Is EERE OBP likely to fund basic/fundamentals algal biology work?
- How can we quickly develop bioinformatics expertise? – i.e., biologists that know computer science or computer scientists that are trained by biologists.



Subtopic #3: Recommendations

Priorities:

- Settle on multiple algal model systems – identification will be dictated by desirable traits
- Ramp up the sequencing of eukaryotic algal genomes as well as cyanobacterial genomes
- Develop an integrated systems biology platform
- Establish a sophisticated genetic toolbox that can be universally applied to different alga.
- Algal research community is small – need to develop the next generation of trained experts as quickly as possible through training programs