Metabolic engineering in microalgae: Challenges in *Dunaliella tertiolecta*

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Eukaryotic microalgae industrial potential

To yield biofuels.
- Oils, e.g. tryacylglycerides (TAG), stored in the cytoplasm/interthylakoid space. Transesterification → biodiesel; distillation+cracking → gasoline/jet fuel.

For molecular farming, heterologous expression of proteins and production of secondary metabolites.
- High growth rates, short generation times.
- The most efficient at converting solar energy.
- Cheap and versatile cultures.
- Good protein post-transcriptional/traductional profiles.

Dunaliella tertiolecta. Differential interference microscopy (1).

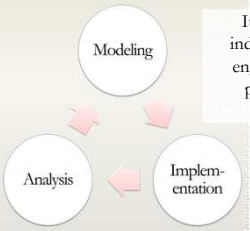
Assets of *D. tertiolecta*

- High growth rate in hypersaline media (↓ contaminations).
- Produces up to 67% lipids and 27% starch (dry weight).
- No rigid wall or theca: easier product extraction and genetic manipulation.
- Accumulates β-carotene: photoprotection and nutraceutical co-product.

Objective

Review the state-of-the-art knowledge to engineer *D. tertiolecta* metabolism in order to design target-directed strategies.

A holistic perspective approach is needed to predict changes in cell biology, e.g. if wanting to know how a lack of some substance or the accumulation of certain by-product would affect cell growth or product yield.

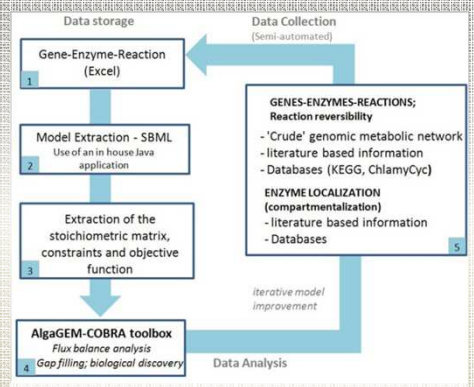


Iterative cycle to achieve industrial goals in microbial engineering, where analysis provides new biological knowlege.

A proper model of 'what would happen if' must be sought to achieve bioengineering goals with less iterations.

In silico modeling of metabolic or cell signaling networks predicts flux variations.

By now, AlgaGEM, a genome-scale metabolic reconstruction based on *C. reinhardtii*, distincts algal behaviours such as catabolism or secretion (currently covering only primary metabolism).



The process for genome-scale model reconstruction, Gomes de Oliveira Dal'Molin *et al* 2011(2).

Genetic transformation methods:
- Particle bombardment (DNA attached to gold shot by gene gun): the best for marine algae and to reach the chloroplast (plastids of *D. tertiolecta* have been successfully transformed).
- Glass beads (good for *D. tertiolecta* thin walls)
- Others: electroporation (also reported in *D. tertiolecta*), microinjection, artificial transposon, recombinant eukaryotic algal viruses, *Agrobacterium tumefaciens*-mediated.

Plastid transformation through homologous recombination.

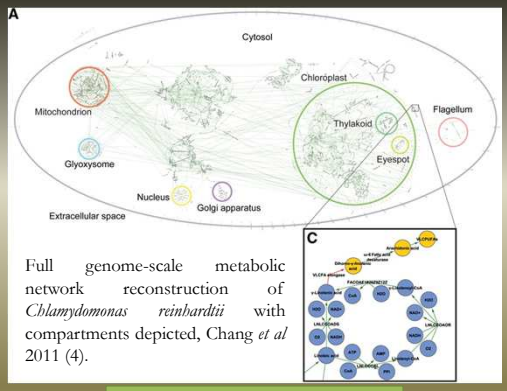
Vector construction:
- Insertion of introns from native genes in heterologous sequences and 3' - 5' UTRs increases protein yield.
- Promoters: DCY1 for stable nuclear transformation in *D. salina*, CaMV35S as universal for marine algal, *D. tertiolecta* induced high heterologous expression with *psbD* promoter and *psbA* terminator, also cab4 light-regulated promoter.
- Codon usage optimization is essential.
- Reporter genes: GFP interfere with algal pigments. X-Gluc.
- Marker genes: chloramphenicol, erythromicin (ereB), bleomycin (ble) inhibited *D. tertiolecta*.
- Gene copy number and homology-dependant gene silencing
- RNA interference technology, detected in *D. salina*.

Synthetic Biology

Systems Biology

"omics" Technology

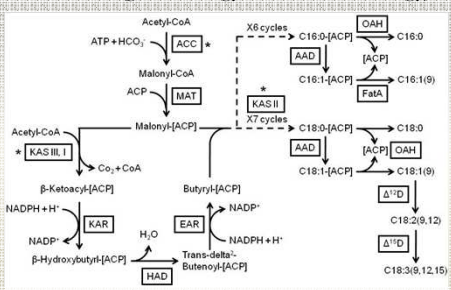
Metabolic Engineering



Full genome-scale metabolic network reconstruction of *Chlamydomonas reinhardtii* with compartments depicted, Chang *et al* 2011 (4).

Genomics:
D. Tertiolecta is not sequenced; but the similar *D. salina* is (NCBI).

Transcriptomics:
Functional genomics. *D. tertiolecta* transcriptome was sequenced under various growth conditions to stimulate reported production and accumulation of lipids or starch: nitrogen limited or sufficient cultures, seawater or salt stressed, and harvesting during log or stationary phases. BLASTx. Functional annotation and pathway identification (gene ontology and KEGG orthology).



One of the schematic pathways reconstructed; fatty acid biosynthesis based on the *de novo* assembly and annotation of *D. tertiolecta* transcriptome, Rismani-Yazdi H *et al* 2011(3).

Improve-import-replace metabolic pathways for

Industrial Biotechnology

Product improvements:
- ↑TAG (ACCase overexpression, polyamine activation of biosynthetic genes, etc.)
- Lipid composition: better properties for biodiesel, shortening lipid chains.

Process improvements:
- Higher iron uptake, ferritin concentration enables magnetic-driven extraction (↓ extraction cost).
- Co-products accumulation such as β-carotene (nutraceutical, food additives).

Conclusions

Genetic tools for *D. tertiolecta* are still scarce to engineer its metabolism. It is essential to sequence its genome for directed genetic engineering and explore its potential.

References

1. Image extracted from: http://eol.org/data_objects/2088133 Authors: David Patterson and Bob Andersen.
2. Gomes de Oliveira Dal'Molin et al. AlgaGEM – a genome-scale metabolic reconstruction of algae based on the *Chlamydomonas reinhardtii* genome. *BMC Genomics* (2011) **12**(Suppl 4):S5.
3. Transcriptome sequencing and annotation of the microalgae *Dunaliella tertiolecta*: Pathway description and gene discovery for production of next-generation biofuels. *BMC Genomics* (2011) **12**:148.
4. Chang RL et al. Metabolic network reconstruction of *Chlamydomonas* offers insight into light-driven algal metabolism. *Molecular Systems Biology* (2011) **7**:518.